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ANALYSIS OF THE *su-leuA* LOCUS IN *SALMONELLA TYPHIMURIUM*

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1. INTRODUCTION

We have been studying a leucine auxotroph of *Salmonella typhimurium*, designated *leu*-151, which when plated on minimal medium spontaneously reverts to wild type and to a type which, in the absence of leucine, grows more slowly than the wild type. Those slow growing reversions have been shown to be due to an additional mutation at a locus linked to the leucine locus. (Smith-Keary 1960). This second mutation partially suppresses the requirement for leucine, and it has been given the symbol *su-leuA*. Thus *su-leuA*-1, *su-leuA*-2 etc. stand for independently isolated suppressors of *leu*-151.

A locus that controls the ability to ferment arabinose (*araB*) is known from transduction experiments to be linked to *leu*. We have previously shown that *su-leuA* is linked to *leu* and that the order is *su-leuA*, *leu*, *araB* (Smith-Keary, 1960). These linkages permit the genetic analysis of independently isolated *su-leuA* mutants and it is possible to show that these mutants specifically suppress *leu*-151 and not some other *leu* mutants and that they are mutations at different sites in the *su-leuA* locus. We have been able to deduce the relevant linkage map of the *su-leuA* region.

2. MATERIALS AND METHODS

The wild type and all the mutants we used were of the LT 2 strain of *Salmonella typhimurium*. Genetic material was transduced with the temperate phage PLT 22, or when sensitive transductants were required, with the H 4 mutant of this phage.

All stocks of *leu*-151 also carried *meA*-22 (requires methionine) and *tryB*-2 (requires tryptophane). In all the relevant experiments adequate amounts of methionine and tryptophane were added to the media and in the following account we shall omit references to these genes and to these requirements.

The mutants *leu*-8, *leu*-10, *leu*-20, *leu*-32, *leu*-38, *leu*-39 and *araB*-9 were obtained from the Carnegie Department of Genetics, Cold Spring Harbor, Long Island, New York.

The preparation of the phage, the method of performing the transductions and the media we used have been previously described (Smith-Keary, 1960). In this account we use the following abbreviations for the different media:

MM	minimal medium.
EMM	minimal medium enriched with broth.
MM+L	media supplemented with 0·002 per cent. dl-leucine.
EMM(arab)+LL	EMM containing 0·2 per cent. arabinose in place of glucose and supplemented with 0·004 per cent. dl-leucine.
EMB	eosin-methylene blue agar.

We characterised the colonies obtained in transduction experiments in one of two ways. When selection was made for colonies that did not require leucine, this was confirmed by streaking or plating each colony on MM and MM+L, and their ability to ferment arabinose was determined by streaking on EMB. When selection was for those colonies that could ferment arabinose, the colonies were first streaked on EMB and, from each streak, a colony that fermented arabinose was streaked or plated on MM and on MM+L to determine whether it required leucine. This sequential streaking is necessary to isolate pure *ara*$^+$ clones from the transductant colonies as these are often a mixture of *ara*$^+$ and *ara*$^-$ cells.

3. EXPERIMENTAL ANALYSIS

Before we describe the genetic analysis of the suppressor locus it is necessary to develop the argument of the previous paper in which the linkage order *su-leuA leu*-151 *leu*-39 *araB*-9 was established so as to decide whether *leu*-151 falls within or without the known limits of the *leu* locus and also whether the *su-leuA* sites are outside or inside these limits. If the *su-leuA* sites are within the *leu* locus we are probably investigating complementation within a locus; if outside we are probably investigating two separate loci.

(i) *Order of* leu *sites*

It is known that *leu*-39 is near the end of the *leu* locus nearest to *araB* and that *leu*-32 is near the end furthest from *araB*. We know that *leu*-151 is further from *araB* than *leu*-39 and we now have to show that it lies between *leu*-32 and *leu*-39. As neither *leu*-32 nor *leu*-151 is available in combination with an *araB* marker the most satisfactory tests (transducing either *leu*-151 *araB*$^-$ with phage raised on *leu*-32 *araB*$^+$ or *leu*-32 *araB*$^-$ with phage raised on *leu*-151 *araB*$^+$, selecting for *leu*$^+$ transductants and scoring for *ara*$^+$ and *ara*$^-$) cannot be made. Instead we used the following methods.

(*a*) *Transductions between* leu-151, leu-32 and leu-39. The frequency of prototrophs from transductions between *leu*-32, *leu*-151 and *leu*-39 in all possible combinations are set out in table 1. That the frequency obtained from the transduction *leu*-39 (×) *leu*-32 is higher than from the transduction *leu*-39 (×) *leu*-151 indicates that *leu*-151 is probably located between *leu*-32 and *leu*-39. The only comparison inconsistent with this conclusion is the higher frequency of prototrophs from the transduction *leu*-151 (×) *leu*-39 than from the transduction *leu*-32 (×) *leu*-39. This inconsistency is probably attributable to the greater effectiveness of *leu*-151 than *leu*-32 as recipient. This greater effectiveness is clearly shown when transductions are made with phage grown on a wild type strain (table 1, last column).

(*b*) *Transductions between* araB-9 *and* leu-32, leu-151 *and* leu-39. Phage grown on each of the leucine mutants was used to infect *araB*-9. *ara*$^+$ transductants were selected by plating on EMM(arab)+LL and these were then tested for their ability to grow in the presence and absence of leucine. The results are set out in table 2. The results of reciprocal transductions, selecting for leucine independence and

testing the transductants for their ability to ferment arabinose, are set out in table 3.

TABLE 1

Wild type transductants per 10^8 survivors in reciprocal transductions between different leu *auxotrophs*

Recipient \ Donor	*leu*-32	*leu*-151	*leu*-39	Wild type
leu-32	0	185	189	3250
leu-151	17·5	0	283	5048
leu-39	617	314	0	2989

TABLE 2

Relative frequencies of leu$^+$ *and* leu$^-$ *transductants from the transductions* araB-9 (×) leu-32, araB-9 (×) leu-151 *and* araB-9 (×) leu-39, *selecting for* ara$^+$ *transductants*

	Donor		
	leu-32	*leu*-151	*leu*-39
leu$^+$	148	100	35
leu$^-$	76	68	81
Total	224	168	116
Per cent. *leu*$^-$	33·93	40·48	69·83

TABLE 3

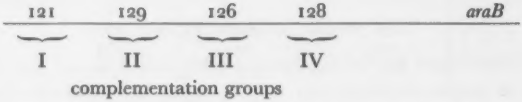
Relative frequencies of ara$^+$ *and* ara$^-$ *transductants from the transductions* leu-32 (×) araB-9, leu-151 (×) araB-9 *and* leu-39 (×) araB-9, *selecting for* leu$^+$ *transductants*

	Recipient		
	leu-32	*leu*-151	*leu*-39
ara$^+$	228	162	145
ara$^-$	135	100	260
Total	363	262	405
Per cent. *ara*$^-$	37·19	38·17	64·20

In both series of experiments the frequency of joint transduction of the selected and unselected marker (per cent. *leu*$^-$ in table 2, per cent. *ara*$^-$ in table 3) was highest with *leu*-39, lowest with *leu*-32 and intermediate with *leu*-151. As the frequency of joint transduction usually decreases with increase in the distance between the markers, though not necessarily proportionately, these results confirm the position of *leu*-151 between *leu*-32 and *leu*-39. Further confirmation

of the relative positions of *leu*-32 and *leu*-151 is provided by the results of the transductions described in section 3.2(*b*).

Recently Margolin has sent us representative mutants of the 4 complementation groups which he has discovered within the *leu* locus: *leu*-121, *leu*-129, *leu*-126 and *leu*-128. Margolin has shown that these complementation groups correspond to the linear arrangement of the sites (Margolin, 1959) and that their linkage order is (personal communication):



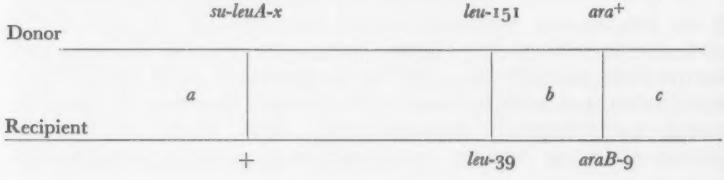
From the results of the transduction *leu*-121 *araB*-9 (×) *leu*-151 and its reciprocal we have found that *leu*-121 is nearer *araB* than is *leu*-151. Thus the order of these sites and *leu*-32 is *leu*-32, *leu*-151, *leu*-121.

No abortive transductions occurred when *leu*-121, as recipient, was transduced with either *leu*-151 or *leu*-32, but these did occur when *leu*-129 or wild type was used as the donor. We conclude that *leu*-151 and *leu*-32 are in the same complementation group as *leu*-121. This is consistent with the linkage data and suggests that there is unlikely to be an additional complementation group to the left of Margolin's group 1.

(ii) su-leuA *and* leu *as separate but linked loci*

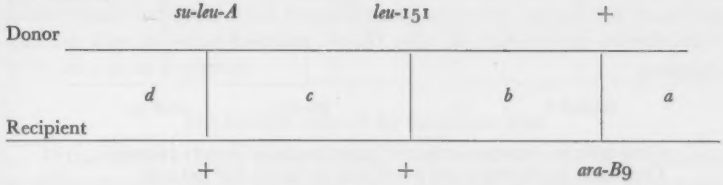
We have already shown that the first isolated suppressor of *leu*-151 (*su-leuA*-1) is linked to *leu* and *araB*. By the following two series of experiments five other independently isolated suppressors have been shown to be linked, and no suppressor of *leu*-151 has been found which is not linked to *leu* and *araB*.

(*a*) *Linkage of suppressors with* leu *and* araB. Phage grown on each of the six suppressed mutants (*su-leuA*-1 *leu*-151, *su-leuA*-2 *leu*-151 etc.) was used to infect *leu*-39 *araB*-9 and selection made for leucine independent transductants. Most of these transductants were slow growing. These slow growing colonies were tested for their ability to ferment arabinose and the frequency of joint transduction of *su-leuA-x* *leu*-151 and *araB*$^+$ showed non-significant variation between 53·1 per cent. and 57·2 per cent. with the different suppressors. Significant variation is not expected as this figure measures the relative frequencies of cross-overs in regions *a* and *b* and in regions *a* and *c* and this is independent of the position of the *su-leuA* site.



These results show that the suppressor site in each of the six suppressed mutants is linked to the *leu* and *araB* loci.

(*b*) su-leuA *and* leu *as separate loci.* Four different suppressed mutants were used as donors in transductions with *araB*-9 as recipient. *ara*+ transductants were selected and then characterised for their ability to grow in the absence of leucine. These transductants must arise, ignoring quadruple cross-overs, by one crossover in region *a* and the second in region *b* (wild type for leucine) or in region *c* (auxotroph for leucine) or in region *d* (suppressed mutant—grows slowly in absence of leucine).



As all three classes can be detected, the results (table 4) provide an estimate of linkage of *su-leuA* with *araB*-9. These results give an average of 45·28 per cent. joint transductions of *leu*-151 and *araB*-9 and an average of 43·67 per cent. joint transductions of *su-leuA* and

TABLE 4

Relative frequencies of fast growing, slow growing and leucine auxotroph transductants from the transduction araB-9 (×) suleuA-x leu-151 araB+ *using different* su-leuA *mutants, selecting for* ara+ *transductants*

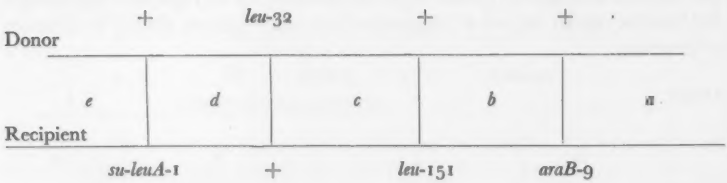
su-leuA allele of donor (*x*)	*ara*+ clones tested	Fast		Slow		Auxotroph	
		Observed	Per cent.	Observed	Per cent.	Observed	Per cent.
1	108	63	58·33	45	41·67	0	0
4	331	184	55·59	139	41·99	8	2·42
2	214	113	52·80	98	45·79	3	1·40
6	89	46	51·69	42	47·19	1	1·12
Totals	742	406	54·72	324	43·67	12	1·62

araB-9. The suppressor locus is thus quite close to *leu*-151. Because of this close linkage it is necessary to consider the possibility that the suppressor sites are within the limits of the leucine locus.

Table 2 shows that there is 33·93 per cent. joint transduction of *leu*-32 with *araB*-9, but, while it is reasonable to take this with other evidence as showing that the order is *leu*-32 *leu*-151 *araB* the data are not sufficiently extensive to decide whether *leu*-32 is relatively far from or very close indeed to *leu*-151. This latter possibility is indicated

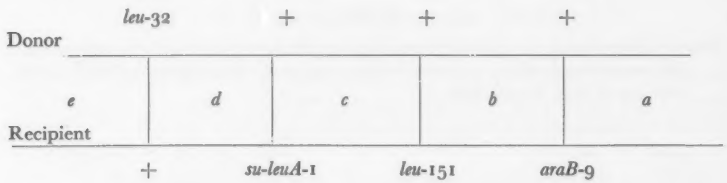
by the similarity in the percentage of joint transductions of *leu*-32 and *araB*-9 and of *leu*-151 and *araB*-9 when selection is for *leu*$^+$ (table 3).

The best available evidence that the suppressor locus is beyond the known limits of the leucine locus, that is further from *araB* than *leu*-32, is from the transduction *su-leuA*-1 *leu*-151 *araB*-9 (×) *leu*-32 using H_4 phage and selecting for *ara*$^+$ transductants. If the order is *su-leuA*-1 *leu*-32 *leu*-151 *araB*-9 this transduction can be represented as follows:



Crossovers in regions *a* and *b* will give *su-leuA*-1 *leu*-151 colonies.
Crossovers in regions *a* and *c* will give *su-leuA*-1 *leu*$^+$ colonies.
Crossovers in regions *a* and *d* will give *su-leuA*-1 *leu*-32 *leu*-151 colonies.
Crossovers in regions *a* and *e* will give *su-leuA*$^+$ *leu*-32.

If the order is *leu*-32 *su-leuA*-1 *leu*-151 *araB*-9 this transduction can be represented as follows:



Crossovers in regions *a* and *b* will give *su-leuA*-1 *leu*-151 colonies.
Crossovers in regions *a* and *c* will give *su-leuA*-1 *leu*$^+$ colonies.
Crossovers in regions *a* and *d* will give *su-leuA*$^+$ *leu*$^+$ colonies.
Crossovers in regions *a* and *e* will give *su-leuA*$^+$ *leu*-32 colonies.

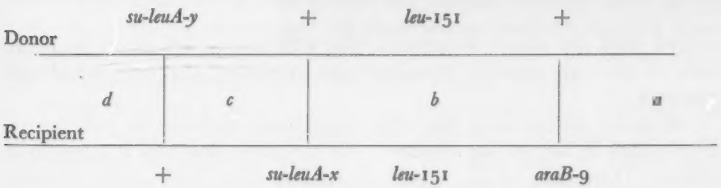
Thus, omitting genotypes arising by quadruple crossovers, all the colonies which grow as wild type in the absence of leucine will, if the first order is correct, be *su-leuA*-1 *leu*$^+$; if the second order is correct they will be either *su-leuA*-1 *leu*$^+$, or, *su-leuA*$^+$ *leu*$^+$. Whether both these genotypes arise can be investigated by taking phage sensitive transductants that grow like wild type in the absence of leucine, raising phage PLT 22 on them, and using this phage to infect *leu*-151. If such a transductant is *su-leuA*-1 *leu*$^+$ both "fast" and "slow" colonies will be produced; five such transductants were scored in this way and all were *su-leuA*-1 *leu*$^+$. Two transductants from a similar cross involving *su-leuA*-4, one from a cross involving *su-leuA*-5 and one from a cross involving *su-leuA*-2 were also found to be *su-leuA*-4 (or 5 or 2) *leu*$^+$.

We conclude that while there remains the slight possibility that *su-leuA* is between *leu*-32 and *leu*-151 (being much closer to *leu*-32 than to *leu*-151) it is more likely that *su-leuA* is outside the known limits of the *leu* locus. This likelihood is increased by the discovery that one of the suppressors (*su-leuA*-1) is a multisite mutant (see next section) and it would seem unlikely that a single site mutant in the leucine locus could be suppressed by such a major disturbance within the same complementation group of the locus.

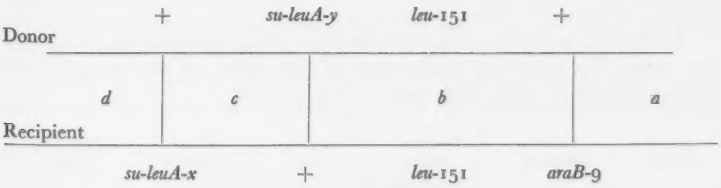
These transductions are further evidence for the relative positions of *leu*-32 and *leu*-151. If *leu*-32 were nearer to *araB* than *leu*-151, the initial transductants would be unlikely to carry the suppressor mutation and no slow growing colonies would arise in the second transduction with *leu*-151 as recipient.

(iii) *Linkage order of the suppressor sites*

If the independently isolated suppressed mutants arise by mutations at different sites within the *su-leuA* locus it should be possible to plot the linkage order of these sites. Consider a transduction *su-leuA-x leu*-151 *araB*-9 (×) *su-leuA-y leu*-151 where *x* and *y* are mutations at different sites of the *su-leuA* locus. If *y* is distal to *x* it should be possible to discover leucine auxotrophs among the *ara*$^+$ transductants: these are unlikely to be discovered if *y* is proximal to *x*.



Those *ara*$^+$ transductants that arise by crossovers in *a* and *c* will be leucine auxotrophs.



Only those very rare transductants that arise by quadruple crossovers in *a* and *b*, and in *c* add *d* will be leucine auxotrophs.

Such an analysis of the six independently isolated suppressed mutants requires that each of these *su-leuA-x leu*-151 genotypes be introduced into the *araB*-9 stock. This was done by transducing

su-leuA-x leu-151 into *leu*-39 *araB*-9 with H4 phage and selecting for transductants that grow in the absence of leucine. From among these phage sensitive, arabinose non-fermenting colonies that only grow slowly in the absence of leucine were chosen. Since *su-leuA-x* does not suppress *leu*-39 (see next section) the strains obtained must be *su-leuA-x leu*-151 *araB*-9.

The proportions of leucine auxotrophs that were found among the *ara*$^+$ transductants when the six suppressed mutants were crossed in all possible ways are set out in table 5. It is conspicuous, and in

TABLE 5

su-leuA-x leu-151 araB-9 (×) su-leuA-y leu-151, *selecting for* ara$^+$ *transductants. Number of leucine auxotrophs among* ara$^+$ *transductants tested*

	su-leuA allele in donor (*su-leuA-y*)					
	1	2	3	4	5	6
su-leuA allele in recipient (*su-leuA-x*) 1	0/100	0/58	0/80	0/90	0/92	0/110
2	0/92	0/75	0/75	1/104	0/105	0/73
3	0/92	1/64	0/60	2/30	1/143	0/80
4	0/103	0/70	0/60	0/60	0/30	0/60
5	0/94	2/82	0/153	8/144	0/30	4/192
6	0/91	1/68	0/192	4/90	0/160	0/130

accord with the above argument, that auxotrophs are never found in both of the two possible transductions involving any two suppressed mutants.

su-leuA-1 differs from the other suppressed mutants in yielding no auxotrophs in any of the crosses. We conclude that this is a multisite mutant (Demerec *et al.*, 1956) extending over the whole distance marked by the mutant sites of the other suppressed mutants. If this were interpreted as a deletion extending close to the end of the *leu* locus it would explain the absence of auxotrophs from the transduction *araB*-9 (×) *su-leuA*-1 *leu*-151 (table 4).

Excluding *su-leuA*-1 and transductions with homologous phage we can make the following deductions of the order of the *su-leuA* sites, with reference to *araB*, from each of the crosses:

su-leuA donor	*su-leuA* recipient	Deduced order	*su-leuA* donor	*su-leuA* recipient	Deduced order
2	3	2-3-*araB*	4	5	4-5-*araB*
2	4	4-2-*araB*	4	6	4-6-*araB*
2	5	5-2-*araB*	5	2	2-5-*araB*
2	6	2-6-*araB*	5	3	5-3-*araB*
3	2	2-3-*araB*	5	4	4-5-*araB*
3	4	4-3-*araB*	5	6	6-5-*araB*
3	5	5-3-*araB*	6	2	2-6-*araB*
3	6	6-3-*araB*	6	3	3-6-*araB*
4	2	4-2-*araB*	6	4	4-6-*araB*
4	3	4-3-*araB*	6	5	6-5-*araB*

With the exception of the transduction where *su-leuA*-6 was in the donor and *su-leuA*-3 in the recipient, from which about two auxotrophs were expected and none found, all the results agree with the order of the sites being:

This order is in full agreement with the crude estimates of the degree of linkage of *su-leuA*-4, *su-leuA*-2 and *su-leuA*-6 with *araB* previously obtained (table 4).

The proportion of auxotrophs from the transduction *su-leuA-x leu*-151 *araB*-9 (×) *su-leuA-y leu*-151 (table 5), which is a rough measure of the distances between the *su-leuA* sites, and the proportion of auxotrophs from the transductions *araB*-9 (×) *su-leuA-x leu*-151 (table 4), which is a rough measure of the distances between the *su-leuA* sites and *leu*-151, are of the same order of magnitude. Thus *su-leuA* and *leu* are probably adjacent loci, and site 151 is close to the end of the *leu* locus nearest the *su-leuA* locus, but not quite as close as *leu*-32.

(iv) *Specificity of suppressors*

We have tested three of the suppressors to discover whether they will suppress other leucine mutants than *leu*-151. The three suppressors tested were *su-leuA*-1, 2 and 5, and the leucine mutants were *leu*-32, 151, 121, 8, 10, 20, 38 and 39. Of these leucine mutants the first three are known to be in the same complementation group; we do not know the complementation groups of the other leucine mutants.

Phage PLT 22 grown on each of 3 stocks separately carrying the 3 mutant suppressors in combination with *leu*$^+$ (obtained from the experiments described in section 3 (ii) (*b*)) was used to infect each of the leucine mutants. Leucine independent transductants were selected and these were examined to discover whether they included any which had slow growth in the absence of leucine. If slow growing transductants were not found we concluded that the *su-leuA* mutant site did not suppress that particular leucine mutant. Slow growing transductants were recovered only when *leu*-151 was infected.

su-leuA-1, 2 and 5 therefore specifically suppress *leu*-151 and do not even suppress other mutant sites in the same complementation group as *leu*-151.

4. NOTE ON METHODOLOGY

We know very little about many of the factors that influence the outcome of a transduction experiment with *Salmonella typhimurium*. For example we are not certain whether all transduced bacterial genetic fragments that include a particular locus are of identical length. The use of some strains as recipient inexplicably results in a larger number of transductants than the use of other strains that are very similar

genetically. We know too little about the degree of negative interference between crossovers to be able to take satisfactory account of this in our predictions. A transduced fragment of bacterial genome may undergo a number of successive incorporations in the recipient cell and its descendants. These complications make us wary of reaching decisive conclusions from particular experiments. For example, the experiments described in section 1 indicate that *leu*-32 is further from *araB* than is *leu*-151 but they do not rule out the possibility that the reverse is true. The additional evidence in section 3 (ii) (*b*), makes the reverse order more unlikely. We can only conclude that, on the available evidence the order *leu*-32 *leu*-151 *araB* is the most likely. All our conclusions are simply the most probable explanations of the data, after ignoring complications that we have no means of assessing.

We would emphasise two technical problems in the present study. First, when leucine-independent transductants are selected by plating on media without leucine, the distinction between slow growing (suppressed mutants) and fast growing (wild type) transductants cannot be made on the transduction plates. Each colony must be picked off and replated on media without leucine so as to give isolated colonies. This involves considerable labour and accounts for some of the data not being more extensive than they are. Second, it is known that a transduced fragment carrying *araB* may undergo successive incorporations in the recipient cell and its descendants. Thus when we select for transduction of *leu*$^+$ and then streak out the colonies to score for arabinose fermentation some may be scored *ara*$^+$ which, if it had been possible to score only the result of the incorporation that introduced the *leu*$^+$, would have been scored *ara*$^-$. There may therefore be in many experiments an excessive proportion of *ara*$^+$ and a deficiency of *ara*$^-$. There is, however, no good reason for supposing that this bias varies significantly from experiment to experiment, as we used the same arabinose marker in all the experiments.

5. DISCUSSION

The *su-leuA*, *leu* and *araB* factors are linked and this has permitted a genetical analysis of the *su-leuA* locus. We have shown that independently isolated suppressed mutants of *leu*-151 arise by mutations at different sites within the suppressor locus. It seems that any of a number of mutations, even a multisite mutation, within *su-leuA* will specifically suppress *leu*-151 and will not suppress seven other *leu* mutants, two of which are in the same complementation group as *leu*-151. It might be thought possible formally to restate these conclusions in terms of complementation and not suppression and say that many mutational changes within *su-leuA* complement *leu*-151. However the term " complementation " is usually reserved for a situation where a wild type phenotype only occurs when either both or neither mutant sites are present, and not when only one is present,

and here the single mutant *su-leuA leu*$^+$ is not an auxotroph but has a nearly wild type phenotype, *su-leuA leu*-151 an intermediate phenotype, and only *su-leuA*$^+$ *leu*-151 is an auxotroph. While it is convenient to maintain this distinction between " suppression " and " complementation " it is by no means certain that the biochemical basis of these phenomena will necessarily always be of a different type. That *su-leuA* and *leu* are probably adjacent regions is perhaps a hint that the biochemical basis of this suppression may be similar to those mechanisms which are invoked to explain complementation within a functional unit. Thus *su-leuA* may be part of the same functional unit as the *leu* locus, having the property that at least some of the possible mutational changes within it only slightly inhibit leucine synthesis and complement a small minority of mutations within the *leu* part of the unit.

Among other microorganisms site specific suppressors are known in *Neurospora* (Giles and Partridge, 1953; Yanofsky and Bonner, 1955) and *Aspergillus* (Käfer, 1958), but in these and other previous studies a genetic analysis has either not been possible or has not been attempted. It may well be that site specific suppression will be found that has its basis in mutation at a single site in a suppressor locus, but the present study has demonstrated that here at least, such suppression arises by many different site, and multisite, mutations within the one locus.

6. SUMMARY

1. Slow growing reversions of *leu*-151 have been shown to be due to mutations at a suppressor locus, *su-leuA*, linked to *leu* and *araB*.

2. *su-leuA* is probably adjacent to and does not overlap the *leu* locus.

3. Five separate sites within the *su-leuA* locus have been plotted. *su-leuA*-1 is probably a multisite mutant.

4. *su-leuA*-1, 2 and 5 suppress *leu*-151 but do not suppress *leu*-8, 10, 20, 32, 38, 39 or 121.

5. A linkage map of the *su-leuA leu araB* region has been constructed and the probable order of the markers shown to be:

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SEGREGATION OF POLYGENES IN ORDERED TETRADS

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1. INTRODUCTION

It is commonly accepted that systems of multiple factors, polygenes, are responsible for most cases of continuous heritable variation. It has been pointed out by Mather (1949) that segregation and linkage are essential features of polygenes. The demonstration of the segregation of polygenes rests on the observed increase of variation in F_2 compared with F_1 generations and the relative variation of these and further generations.

Previous work (Pateman, 1955; 1959; Lee and Pateman, 1959) has shown that size of ascospore in *Neurospora crassa* is controlled by a polygenic system. The experiments reported here provide a direct demonstration of the segregation of polygenes affecting ascospore size during the formation of ordered tetrads (asci) in *N. crassa*.

The majority of the life cycle of *N. crassa* is haploid. It is desirable to clarify the use of such terms as P_1, F_1 and B_1 when applied to the stages of such a life cycle. Figure 1 shows the degree of ploidy of all

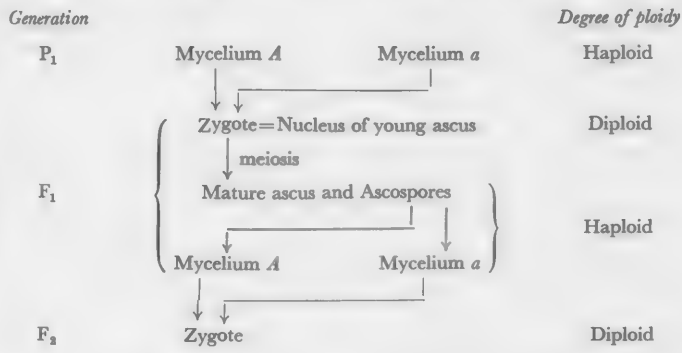

FIG. 1.—Diagrammatic illustration of life cycle of *N. crassa*.

relevant stages and the generation to which they are considered to belong. This usage is followed throughout this paper. A first backcross generation, B_1 is derived from a cross between an F_1 mycelium and a P_1 mycelium. A B_2 generation is derived from a cross between a B_1 mycelium and a P_1 mycelium.

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2. METHODS

The detailed origin of strains 232-2, 232-4, and St.L.*A* was described by Pateman (1959). Strains 232-2 and 232-4 were obtained from a cross between two strains at the fifteenth generation of selection for increased ascospore size. The mean length of ascospores from the cross 232-2 × 232-4 was 17·76, and the mean length of ascospores from a wild-type cross was 13·55, the unit of measurement being about 2·17μ. That is, the genotype built up by selection in strains 232-2 and 232-4 resulted in an increase of about 35 per cent. in mean ascospore length compared with wild-type.

In the present work a projection microscope was used to measure the ascospores, the unit of measurement being approximately 1·73 μ. Vegetative cultures were maintained on agar slopes of Fries No. 3 medium (Beadle and Tatum, 1945). All crosses were made on agar slopes of a medium favouring sexual reproduction (Westergaard and Mitchell, 1947) and incubated at 25° C.

3. RESULTS

It was not possible to demonstrate satisfactorily segregation of polygenes during the formation of F_1 asci from the cross 232-4 × wild-type, since F_1 ascospores are phenotypically similar to wild-type. An attempt to demonstrate polygenic segregation using crosses between the members of ascus 232 was unsuccessful, partly because any genotypic differences were small in comparison with environmental variation (Pateman, 1959). Consequently, asci from backcrosses to the large-spored strain 232-4 were used to demonstrate such segregation. In the formation of backcross asci the ratio of large-spored genotype to wild genotype was 3 : 1. When members of the backcross asci were again crossed to the large-spored strains 232-2 or 232-4 the ratio of large-spored genotype to wild was 7 : 1. This preponderance permitted the phenotypic expression of the large-spored genotype. Also the presence of a small proportion of wild genotype reduced the environmental variation between replicate crosses.

The derivation of the backcross asci and the testcrosses were as follows. Three single ascospore, F_1 cultures designated 1, 2 and 3 were obtained from the cross 232-4 × St.L. From the backcross 1 × 232-4, ascus 1 was dissected and the eight single ascospore cultures 1-1 to 1-8 obtained. From the backcross 2 × 232-4 the three asci 2, 3 and 4 were obtained. From the backcross 3 × 232-4, asci 5 and 6 were obtained. Members of each of the six asci were crossed to either 232-2 or 232-4; the implications of the use of two " tester " strains are discussed later. Five replicates of each cross were made and fifty ascospores from each replicate were measured. The mean length of each sample of ascospores provided an estimate of the genotype of the member of the ascus. In the case of the two asci 1 and 5, all eight members of each were testcrossed in this way. With the other four asci only one member of each mitotic pair was testcrossed. This still permitted comparisons between each of the four meiotic products in each ascus. The results of these crosses are given in table 1. In all six asci the segregation of the mating type alleles *A* and *a* is given. In asci 1, 5 and 6 the segregation is also given of two alleles at the

TABLE 1

Mean lengths of samples of fifty ascospores taken from replicate testcrosses of members of asci 1 to 6

Ascus 1	Replicates					$\bar{x}$
1-1 *al*$^+$ *A*	19·06	19·10	18·54	19·08	19·00	18·96
1-2 *al*$^+$ *A*	18·72	17·96	18·32	19·00	19·10	18·62
1-3 *al* *a*	20·86	20·24	20·26	20·32	20·04	20·34
1-4 *al* *a*	21·04	20·00	20·44	19·96	20·04	20·30
1-5 *al* *A*	18·66	18·06	18·40	18·92	18·64	18·54
1-6 *al* *A*	18·52	18·44	19·18	18·56	19·02	18·74
1-7 *al*$^+$ *a*	22·90	21·92	21·62	21·20	22·38	22·00
1-8 *al*$^+$ *a*	22·14	21·76	21·08	21·20	21·64	21·56

Ascus 2	Replicates					$\bar{x}$
2-2 *A*	19·00	18·72	19·04	19·08	19·08	18·98
2-4 *a*	18·68	18·56	18·56	18·56	18·52	18·58
2-6 *a*	19·74	19·46	19·50	19·84	19·88	19·68
2-7 *A*	20·46	19·94	20·40	20·10	20·14	20·21

Ascus 4	Replicates					$\bar{x}$
4-2 *a*	24·14	24·34	24·32	24·30	24·14	24·25
4-4 *A*	20·12	20·10	20·12	20·10	20·08	20·10
4-6 *A*	20·76	20·68	20·96	20·66	20·58	20·73
4-7 *a*	21·86	22·00	21·32	21·84	22·04	22·81

Ascus 5	Replicates					$\bar{x}$
5-1 *al*$^+$ *A*	17·72	17·60	17·68	17·68	17·62	17·66
5-2 *al*$^+$ *A*	17·68	17·56	17·64	17·60	17·68	17·63
5-3 *al* *A*	19·24	19·08	18·98	18·94	19·14	19·08
5-4 *al* *A*	19·06	18·96	19·10	18·96	19·00	19·02
5-5 *al* *a*	23·92	23·98	24·02	23·88	23·94	23·95
5-6 *al* *a*	23·96	23·96	23·78	23·90	23·96	23·91
5-7 *al*$^+$ *a*	22·88	23·02	22·88	23·04	23·02	22·97
5-8 *al*$^+$ *a*	22·94	23·08	22·90	22·88	23·08	22·98

Ascus 3	Replicates					$\bar{x}$
3-2 *a*	19·60	19·64	19·60	19·62	19·70	19·63
3-4 *a*	23·34	23·46	23·36	23·56	23·72	23·49
3-6 *A*	22·58	22·26	22·10	22·56	22·12	22·32
3-7 *A*	19·06	18·92	18·90	18·92	19·00	18·96

Ascus 6	Replicates					$\bar{x}$
6-2 *al* *a*	21·80	22·16	21·58	22·04	21·06	21·84
6-4 *al*$^+$ *A*	23·70	23·56	23·44	23·58	23·50	23·56
6-6 *al*$^+$ *a*	17·70	17·78	17·76	17·72	17·66	17·72
6-7 *al* *A*	17·94	17·94	18·04	18·14	17·94	18·00

albino locus, al^+ and *al*. Both the mating type and albino alleles show the expected 2 : 2 segregation in each ascus. Those strains in each ascus carrying the *a* allele were testcrossed to 232-2 *A* and those carrying the *A* allele were testcrossed to 232-4 *a*.

Inspection of table 1 shows that there was little variation between replicates or between the means of strains from the same mitotic pair. By comparison there were large differences between some strains which were derived from different meiotic products within the same ascus.

4. STATISTICAL ANALYSIS

(i) *Segregation and recombination of polygenes*

The analyses of variance of the testcross data are given in table 2. There are seven degrees of freedom for comparisons between the eight members of asci 1 and 5. There are several ways in which the seven degrees of freedom may be partitioned to give orthogonal sets of comparisons which have some biological significance. The most useful set is that given in table 2. This gives a comparison between the members of each mitotic pair, a comparison between the two halves of the ascus and two comparisons between different meiotic products. Similarly the most useful set of orthogonal comparisons utilising the three degrees of freedom available in asci 2, 3, 4 and 6 is given in table 2. The analyses show that there were no significant differences between any two members of the same mitotic pair. But there were significant differences in most of the comparisons between different meiotic products within the same ascus. This demonstrates that segregation of polygenes effecting ascospore length occurred during the six meiotic divisions which resulted in asci 1 to 6.

In the analyses of variance the number of comparisons possible was limited by the degrees of freedom available and the necessity that the comparisons should be orthogonal. It would be preferable if comparisons between the two members of each mitotic pair and all the possible comparisons between different meiotic products of an ascus could be made. It is possible to make all the desirable comparisons within each ascus by a series of *t* tests. The results of such tests are given in table 3. The *t* tests indicate that there was no significant difference between members of the mitotic pair, but in the majority of cases the difference between different meiotic products was significant.

There is some doubt concerning the independence of members of a multiple set of *t* tests such as those given in table 3. A number of authors have proposed methods for dealing with this kind of problem. In particular, Duncan (1955) has developed a " new multiple range test " which, it is claimed, enables independent comparisons to be made between all members of a set of means. The results of the application of this method are given in table 4. The conclusions which can be drawn from this method are in close agreement with those of the analyses of variance and the *t* tests.

TABLE 2

Analyses of variance of testcross data from table 1

Item	Ascus 1				Ascus 5			
	n	m.s.	F	P	n	m.s.	F	P
1 *v.* 2	1	14·10	1·6	>0·2	1	0·1	0·007	>0·2
3 *v.* 4	1	0·97	0·01	>0·2	1	0·4	0·03	>0·2
5 *v.* 6	1	5·4	0·61	>0·2	1	0·2	0·01	>0·2
7 *v.* 8	1	24·20	2·7	>0·05	1	0·002	0·000015	>0·2
(1+2+3+4) *v.* (5+6+7+8)	1	223·0	25·3	>0·001	1	13025	950·7	>0·001
(1+2) *v.* (3+4)	1	571·5	64·9	<0·001	1	490	35·8	<0·001
(5+6) *v.* (7+8)	1	2471	280·7	<0·001	1	230	16·8	<0·001
Between strains	7	474·3	53·8	<0·001	7	1964	143·3	<0·001
Between replicates	32	8·8	2·2	<0·001	32	13·7	1·34	<0·05
Total between crosses	39	92·39	10·5	<0·001	39	364	26·5	<0·001
Error	1960	4·0	—	—	1960	10·2	—	—
Total	1999	—	—	—	1999	—	—	—

Item	Ascus 2				Ascus 3			
	n	m.s.	F	P	n	m.s.	F	P
(2+4) *v.* (6+7)	1	154·4	35·9	<0·001	1	210·7	20·5	<0·001
2 *v.* 4	1	10·4	2·4	>0·05	1	1859·0	180·5	<0·001
6 *v.* 7	1	17·2	4·0	<0·05	1	1415	137·3	<0·001
Between strains	3	131·6	30·6	<0·001	3	1161·0	112·7	<0·001
Between replicates	16	1·4	3·1	<0·01	16	1·1	9·4	<0·001
Total between crosses	19	21·9	5·1	<0·001	19	184·0	17·9	<0·001
Error	980	4·3	—	—	980	10·3	—	—
Total	999	—	—	—	999	—	—	—

Item	Ascus 4				Ascus 6			
	n	m.s.	F	P	n	m.s.	F	P
(2+4) *v.* (6+7)	1	183·2	13·7	<0·001	1	5841·0	758·6	<0·001
2 *v.* 4	1	2147·0	160·2	<0·001	1	369·8	48·0	<0·001
6 *v.* 7	1	175·2	13·1	<0·001	1	9·5	1·2	>0·2
Between strains	3	835·0	62·3	<0·001	3	2074·0	269·4	<0·001
Between replicates	16	0·3	43·2	<0·001	16	1·7	4·5	<0·001
Total between crosses	19	132·0	426·0	<0·001	19	328·0	42·6	<0·001
Error	980	13·4	—	—	980	7·7	—	—
Total	999	—	—	—	999	—	—	—

TABLE 3

Comparisons of mean ascospore lengths obtained from testcrosses of members of asci 1 to 6. There are ten comparisons each for asci 1 and 5, since all eight members of each were testcrossed. There are six comparisons each for asci 2, 3, 4 and 6

Ascus 1

Comparison	1 *v.* 2	3 *v.* 4	5 *v.* 6	7 *v.* 8	(1+2) *v.* (3+4)	(1+2) *v.* (5+6)	(1+2) *v.* (7+8)	(3+4) *v.* (5+6)	(3+4) *v.* (7+8)	(5+6) *v.* (7+8)
t_8 . .	1·41	0·16	0·96	1·25	8·94	0·92	13·5	10·74	6·76	15·01
P . .	>0·1	>0·8	>0·3	>0·2	<0·001	>0·4	<0·001	<0·001	<0·001	<0·001

Ascus 5

Comparison	1 *v.* 2	3 *v.* 4	5 *v.* 6	7 *v.* 8	(1+2) *v.* (3+4)	(1+2) *v.* (5+6)	(1+2) *v.* (7+8)	(3+4) *v.* (5+6)	(3+4) *v.* (7+8)	(5+6) *v.* (7+8)
t_8 . .	0·88	0·98	0·94	0·18	40·87	201·46	171·84	130·52	97·09	27·99
P . .	>0·2	>0·3	>0·3	>0·8	<0·001	<0·001	<0·001	<0·001	<0·001	<0·001

	Ascus 2		Ascus 3		Ascus 4		Ascus 6	
Comparison	t_8	P	t_8	P	t_8	P	t_8	P
2 *v.* 4 . .	5·46	<0·001	53·27	<0·001	83·08	<0·001	13·88	<0·001
2 *v.* 6 . .	6·38	<0·001	25·42	<0·001	40·32	<0·001	34·93	<0·001
2 *v.* 7 . .	11·26	<0·001	18·76	<0·001	17·91	<0·001	31·34	<0·001
4 *v.* 6 . .	12·09	<0·001	9·35	<0·001	9·76	<0·001	119·46	<0·001
4 *v.* 7 . .	17·91	<0·001	59·35	<0·001	13·44	<0·001	114·43	<0·001
6 *v.* 7 . .	3·81	<0·01	30·99	<0·001	7·49	<0·001	6·17	<0·001

* For the six comparisons in each of asci 1 and 5 where the mitotic pairs are combined n=18.

In effect the crossing procedure described means that the genotypes of the members of an ascus are assayed against the genotypic background of the tester strains 232-2 and 232-4. It was necessary to use two tester strains of different mating type. *Neurospora crassa* is heterothallic and the mating type alleles *A* and *a* segregated 1 : 1 in each ascus. Ideally, the tester strains should have been isogenic except for the mating type alleles. In practice, strains 232-2 and 232-4 were the best available.

TABLE 4

Analysis by "new multiple range test" of differences between strain means in asci 1 to 6. Any two means not underscored by the same line are significantly different at 1 per cent. level

	Ascus 1							
Strain number	7	8	3	4	1	6	2	5
Strain means ranked in order	22·00	21·56	20·34	20·30	18·96	18·74	18·62	18·54
	Ascus 5							
Strain number	5	6	8	7	3	4	1	2
Strain means ranked in order	23·94	23·91	22·97	22·96	19·07	19·01	17·66	17·63
	Ascus 2				Ascus 3			
Strain number	7	6	2	4	4	6	2	7
Strain means ranked in order	20·21	19·68	18·98	18·58	23·48	22·32	19·63	18·96
	Ascus 4				Ascus 6			
Strain number	2	7	6	4	4	2	7	6
Strain means ranked in order	24·24	21·81	20·72	20·10	23·56	21·83	18·00	17·72

Samples of ascospores were measured from backcrosses to the large-spored parent of 187 F_1 strains obtained from crosses between 232-2 and 232-4 and stock wild-types (B. T. O. Lee, unpublished). The results showed that there were no significant genotypic differences between 232-2 and 232-4 with respect to ascospore size. If, however, there was such a genotypic difference between 232-2 and 232-4 it could not have been responsible for all of the observed differences between the testcrosses of members of the same ascus. From each ascus two of the meiotic products were testcrossed to 232-2 and the other two to 232-4. Thus it is possible to compare two members of an ascus which have been testcrossed to the identical strain. There

are twelve such comparisons possible, two in each of the six asci. For example, 3-2 × 232-2 versus 3-4 × 232-2 and 3-6 × 232-4 versus 3-7 × 232-4 from ascus 3. It can be seen from the multiple range test of all six asci that eleven of the twelve possible comparisons are significant at the 1 per cent. level. In none of these eleven comparisons could the observed significant differences be due to any difference between 232-2 and 232-4. The differences must have been due to genotype differences between different meiotic products within the same ascus.

(ii) *Interaction of polygenes*

Asci 5 and 6 were derived from the same cross and asci 2, 3 and 4 were derived from the same cross. This means that asci 5 and 6 were derived by separate meiotic divisions from identical diploid nuclei and asci 2, 3 and 4 were also the result of separate meiotic divisions of identical diploid nuclei. If the individual effects of the members of this polygenic system were simply additive, then the mean of the four meiotic products of one diploid nucleus should be approximately equal to the mean of the meiotic products of a second identical diploid nucleus. But the differences between the four meiotic products of a diploid nucleus will depend on the particular combinations of polygenes carried by each meiotic product. It was possible to make four such comparisons between the combined means of the meiotic products from identical nuclei: ascus 2 versus ascus 3, 2 versus 4, 3 versus 4 and 5 versus 6. The analyses of variance which enabled the appropriate comparisons to be made are given in table 5. It can be seen that the differences between the asci in all four comparisons are significant. Therefore the polygenes in this system interact in their effects on ascospore length. The magnitude of this interaction may be quite large. The mean ascospore length of the largest member of ascus 2 was approximately equal to that of the smallest member of ascus 4. The mean ascospore length obtained from all members of ascus 2 was 19·36, the mean length from all members of ascus 4 was 21·72, a difference of 2·36.

5. DISCUSSION

The experiments described showed that there was no detectable genetic difference with respect to ascospore length in strains which were the result of a single mitosis. In comparison there were many instances of large and significant genetic differences between strains which were derived from different products of a single meiotic division. This behaviour of the genetic material effecting ascospore length parallels that of the Mendelian alleles, *a*, *A* and *al*, *al*$^+$, which were also segregating during the formation of the ascospores. It is clear that the polygenes concerned segregated and recombined during single meiotic divisions in a similar fashion to Mendelian genes. This confirms the view first proposed by Nilsson-Ehle (1909), East (1915)

TABLE 5

Comparisons between complete asci derived from identical parents

Item	Asci 2 and 3				Asci 2 and 4				Asci 3 and 4				Asci 5 and 6			
	n	m.s.	F	P	n	m.s.	F	P	n	m.s.	F	P	n	m.s.	F	P
Between ascus means	1	1510	206·8	<0·001	1	2844	319·6	<0·001	1	209·3	17·7	<0·001	1	139·1	15·6	<0·001
Other strain comparisons	6	647·2	88·7	<0·001	6	483·3	54·3	<0·001	6	998·3	84·6	<0·001	6	2185	245·5	<0·001
Between strains	7	770·4	105·5	<0·001	7	820·6	92·2	<0·001	7	885·6	75·1	<0·001	7	1893	212·7	<0·001
Between replicates	32	1·03	7·1	<0·001	32	0·97	9·2	<0·001	32	1·06	11·1	<0·001	32	0·75	11·9	<0·001
Between crosses	39	139·1	19·1	<0·001	39	148·1	16·6	<0·001	39	159·8	13·5	<0·001	39	340·4	38·2	<0·001
Error	1960	7·3	—	—	1960	8·9	—	—	1960	11·8	—	—	1960	8·9	—	—
Total	1999	—	—	—	1999	—	—	—	1999	—	—	—	1999	—	—	—

and developed by Fisher (1918) and Mather (1949). Namely, continuous quantitative variation can be explained by genetic factors of small individual effects which segregate and recombine in a similar fashion to major genes. Previous experimental demonstration of the segregation of multiple factors or polygenes was indirect. It depended essentially on the relative variation of different generations in certain breeding programmes, notably the usually observed increase of variance in the F_2 generation.

The phenotypic effects of polygenes and Mendelian genes differ in magnitude, but there is no reason to suppose that this is due to any fundamental differences in the characteristics of the genetic material of which they are composed. It is probable that polygenes and major genes represent opposite ends of a continuous spectrum of possible gene effect. In the polygenic system effecting ascospore length in *Neurospora crassa* the phenomena of dominance, (Pateman, 1959), linkage (Lee and Pateman, 1959) and in the present work segregation, recombination, and gene interaction have all been demonstrated. This evidence supports the hypothesis that polygenic and major gene variation are fundamentally similar in nature.

6. SUMMARY

1. It has been established previously that the genetic determination of ascospore length in *Neurospora crassa* is chiefly polygenic. From a cross between a normal strain and a strain whose genotype was known to determine increased ascospore length, F_1 strains were obtained. From backcrosses between F_1 strains and the large-spored strain, six complete asci were isolated and dissected. The member strains of each ascus were backcrossed to the large-spored strain and the mean length of samples of the ascospore progeny obtained. The second backcrosses provided an estimate of the genotypes, with respect to the determination of ascospore length, of each single ascospore strain from each ascus.

2. Analysis of the backcross data enabled the following conclusions to be drawn: There were no significant differences, with respect to mean ascospore length, between strains derived from a single mitotic division. In many instances there were significant differences, with respect to mean ascospore length, between strains derived from different products of the same meiotic division. There were significant differences, with respect to mean ascospore length, between the total means obtained from separate asci, where each ascus had been derived by a single meiosis from identical diploid nuclei.

3. The results provided a direct demonstration of the segregation and recombination of polygenes during single meiotic divisions. They also showed that the phenotypic effects of the polygenes effecting ascospore length were not simply additive, there was a considerable amount of gene interaction.

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MUTAGENIC SENSITIVITY OF SPERM, SPERMATIDS, SPERMATOCYTES AND SPERMATOGONIA IN *DROSOPHILA MELANOGASTER*

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1. INTRODUCTION

LÜNING (1952*a*), measuring dominant lethal incidence in successive days' matings following a single dose of X-rays in *Drosophila melanogaster*, found a variation in sensitivity in post-meiotic stages, which he confirmed by his work with hyperploids (1952*b*). This variation was attributed to hypersensitivity of the early post-meiotic stages, that is, spermatids. Auerbach (1954), using three-day " brood " periods and testing for recessive autosomal and sex-linked lethals found a peak mutational response in the second brood *i.e.* 4-7 days after irradiation (which she equated to spermatids at irradiation). This was followed by a period of high sterility in the third brood.

This hypersensitivity of spermatids has now been established in *D. melanogaster* for dominant lethals (Lüning, 1952*a*, Bateman, 1956); hyperploids (Lüning, 1952*b*, Bateman, 1957); autosomal and sex-linked lethals (Auerbach, 1954, Oster, 1955, 1956.); and translocations (Oster, 1955, 1956); and also in *D. virilis* for dominant lethals and translocations (Alexander and Stone, 1955).

In the " brood pattern " technique of Auerbach (1954) where each brood is the product of three days' matings, the mutation rates measured will represent average effects in what may often be heterogeneous material. Furthermore, the contribution of each day's mating to a brood will be weighted according to its fertility which itself will be expected to vary systematically, and thus a bias will be introduced into the overall brood result.

In the present study, it was desired to determine whether the changes in sensitivity were the same for different sectors of the mutation spectrum. For this purpose, dominant lethals, deleted X's, and translocations (all structural changes), autosomal and sex-linked lethals (mainly gene changes) were followed in the same material. At the same time evidence of induced crossing-over in the *b pr vg* region of chromosome II was obtained, primarily for its value in identifying spermatogenic stages, though it proved to have a value in its own right.

It would have been cumbersome to follow *daily* changes in all these mutations, so four days were selected as being most representative. The second day after irradiation of the male showed the minimum sensitivity of sperm to dominant lethals. The eighth day had shown

the maximum sensitivity for deleted X's and was the last day for high dominant lethal rate. The eleventh day showed a return to control values for deleted X's and dominant lethals and almost certainly represented spermatogonia surviving irradiation. The fifth day was chosen as this was half-way in time between the second and eighth.

2. MATERIALS AND METHODS

The males which were used throughout the experiment were the F_1 hybrid between Birmingham Outbred females and *b pr vg* males.

For each run, 25 F_1 males which had emerged overnight were irradiated in the morning with 1000r X-rays (a Resomax X-ray tube run at 300 kV was used for all exposures). Immediately after treatment they were mated to F_1 females at the rate of two per male per day. This mating rate was used throughout the course of the experiment and was chosen in order to ensure that sperm was used as it matured and was not stored (Bateman, 1956) a situation which would lead to the mixing of successive batches of sperm and confuse the sensitivity pattern of the several spermatogenic stages.

On day 2, which was the first sampling day, the males were divided into five equal groups A-E, the five males in each group being allocated to a different type of female in the ratio of two females to each male.

Group A males were mated to Muller-5 females for the detection of sex-linked recessive lethals.

Group B males were mated to $\frac{CyL}{Pm}$, $\frac{H}{Sb}$ stock females for the estimation of autosomal recessive lethals and translocations. Group C males were back-crossed to *b pr vg* females in order to detect induced crossing-over.

Group D males were mated to F_1 females and the rate of dominant lethals was scored as the percentage non-hatching eggs (using the technique of Bateman, 1956). The dominant lethal rate was used to check that each batch of males was conforming to the usual pattern in sensitivity and rate of spermatogenesis. An unirradiated control series was run concurrently.

In Group E, the males were mated to *y v f car* attached—X females for the determination of deleted X's, which were recognised in the progeny as non-yellow (*i.e.* hyperploid) daughters.

This procedure was repeated on each of the other sampling days, 5, 8 and 11. Sometimes, during the periods between the main sampling days, the males were mated to *b pr vg* females instead of F_1 females in order to follow the incidence of induced crossing-over. In this way, the daily frequency of crossing-over was obtained from day 2 up to and including day 13.

Successive runs of the experiment were made until sufficient data had been obtained for our purposes.

In estimating standard errors for some of the mutation rates we have followed the advice of Stevens (1942) in allowing for the skewness of probability distributions at low frequencies. Instead of the conventional $\pm$ symbol for the standard error we have quoted separately the upper and lower limits at the probability level of 0·15, which is the level corresponding to the standard error.

3. RESULTS

(i) *Dominant lethals*

Table 1 shows the percentages of unhatched eggs on the four sampling days for control and treated males. From this the reader can judge the consistency of the ten successive runs. The overall mean percentages are also given with their standard errors based upon

variation between runs. Fig. 1*a* shows the variation in dominant lethal rate graphically for both irradiated males and unirradiated controls, with the results of Bateman (1956) for comparison. The incidence of dominant lethals rises sharply from day 2 to day 5 and rises further to a peak on day 8. This second rise could be spurious,

TABLE 1

Percentage of non-hatching eggs following 1000r X-rays to males

Day		Run										Mean per cent.
		1	2	3	4	5	6	7	II	9	10	
2	Control	—	1·76	0·33	0·93	12·30	0·23	9·93	0·82	0·89	4·90	3·56
	Irrad.	8·01	15·34	13·60	9·50	14·10	8·19	9·05	9·52	9·14	9·40	10·59±0·85
5	Control	2·04	0·88	0·83	0·25	1·33	0·69	0·54	0·78	0·72	0·50	0·86
	Irrad.	48·13	29·55	41·70	31·95	27·00	59·84	53·90	43·97	34·40	40·50	41·09±3·39
8	Control	0·80	1·89	1·40	1·00	5·90	0·00	0·83	2·42	0·33	3·40	1·80
	Irrad.	56·61	60·30	72·40	62·60	61·00	58·00	59·40	56·14	57·10	67·30	61·09±1·64
11	Control	1·84	2·10	0·40	1·32	0·84	0·00	0·85	0·57	0·98	0·40	0·93
	Irrad.	1·37	15·80	1·20	0·14	4·70	2·10	1·59	0·50	8·30	5·40	4·11±1·53

however, if the non-hatching percentages contained a large number of unfertilised eggs. Day 11 shows a drop to a value just slightly higher than the control level.

As stated previously, the dominant lethal incidence was followed mainly as a check on the running conditions of the experiment, but also fully confirms earlier observations.

(ii) *Deleted X's*

Table 2 gives the hyperploid frequency obtained over the four sampling days following 1000r X-rays. These data, though not very

TABLE 2

Percentage Hyperploidy following 1000r X-rays to males

Day	2	5	II	11
Total *y v f car* ♀♀	2532	1344	286	1011
Total hyperploid ♀♀	3	3	6	0
Per cent. hyperploids	0·12	0·22	2·10	0·00
SE upper limit	0·24	0·45	3·32	0·18
SE lower limit	0·06	0·10	1·22	0·00

extensive, fully confirm the peak incidence at day 8 found earlier (Bateman, 1957). The results are expressed graphically in fig. 1*b*.

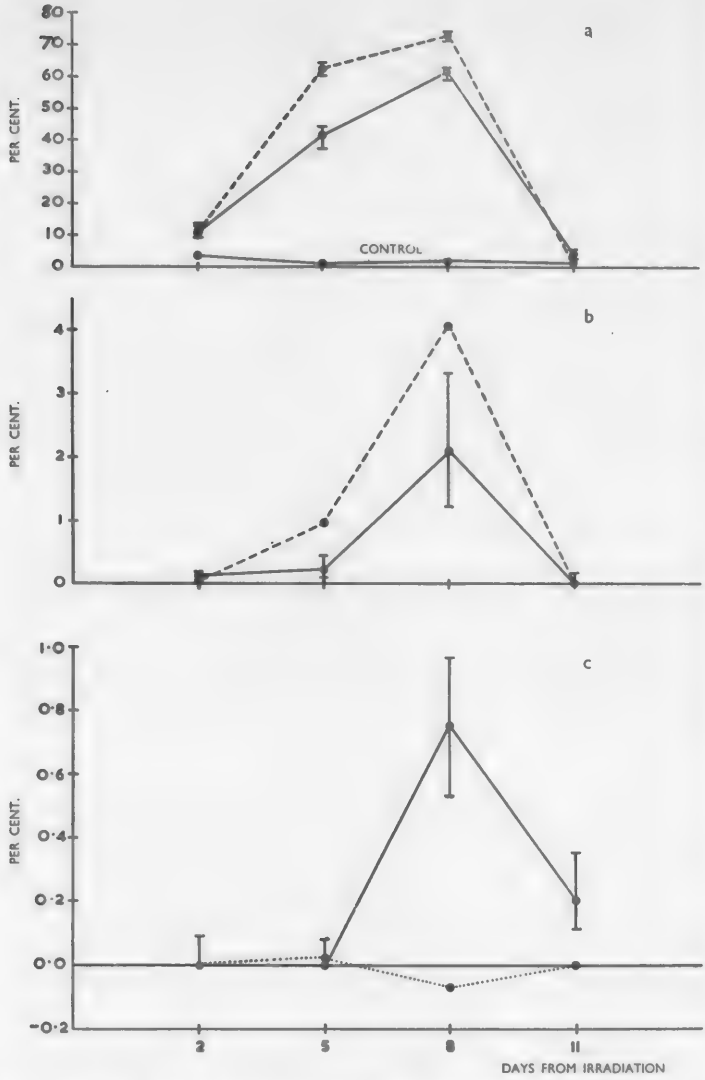

FIG. 1.—Variation in the rate of (*a*) Dominant lethals, (*b*) Hyperploids and (*c*) Cross-overs and "Mutations" on days 2, 5, 8 and 11 following 1000r X-rays. In graphs (*a*) and (*b*) continuous lines represent present data. Broken lines represent data of Bateman (1956, 1957). In graph (*c*) continuous line represents per cent. cross-overs and dotted line represents per cent. "mutation" from present data.

Standard errors are represented by vertical lines.

(iii) *Autosomal recessive lethals*

The data on the frequency of recessive autosomal lethals are given in table 3 and fig. 2*a*.

The highest mutation rate was found on day 5 (12·19 per cent.). On day 8, the day showing the highest rate of both dominant lethals and deleted X's, the level of autosomal lethals was definitely lower (7·95 per cent.), indeed half-way between the values for day 5 and day 11, (3·82 per cent.) which showed a mutation rate even lower than day 2 (5·12 per cent.).

Analysis of the data obtained on the four days showed a highly significant variation between days. The standard errors were calculated

TABLE 3

Percentage autosomal and sex-linked recessive lethals following 1000*r X-rays to males*

Day	2	5	8	11
Autosomals				
Total . . .	781	607	566	680
Lethal . . .	40	74	45	26
Per cent. lethal .	5·12±0·79	12·19±1·33	7·95±1·14	3·82±0·74
Sex-linked				
Total . . .	944	617	440	731
Lethal . . .	19	35	15	9
Per cent. lethal .	2·01±0·46	5·67±0·93	3·41±0·87	1·23±0·41
Ratio autosomal/ sex-linked . .	2·5	2·1	2·3	3·1

from the total sample sizes on each day assuming homogeneity between successive runs of the experiment.

The results are in agreement with those of Auerbach (1954) who found a significant increase in mutation rate following 2000r X-rays, in the second brood, that is matings 4-7 days after treatment. In the tests for autosomal lethals however, Auerbach found excessive sterility in the treated males in the second brood. This she attributed to a speeding up of the rate of sperm release by the use of *Cy L* females, so that treated spermatocytes were already being utilised in the second brood. In all our matings, however, this period of sterility was confined to the single day 8. The difference may be due to the different dose levels (2000 and 1000r respectively).

(iv) *Sex-linked lethals*

The frequency of sex-linked recessive lethals is shown in table 3 and fig. 2*b*. There is a striking parallelism with the variation in autosomal lethal incidence. Statistical analysis of the results showed a highly significant variation between days with no detectable heterogeneity

between runs. The standard errors were based on the total sample sizes for each day. The reduced yield of sex-linked lethals from later matings of irradiated males has generally (*e.g.* Auerbach, 1954, Khishin,

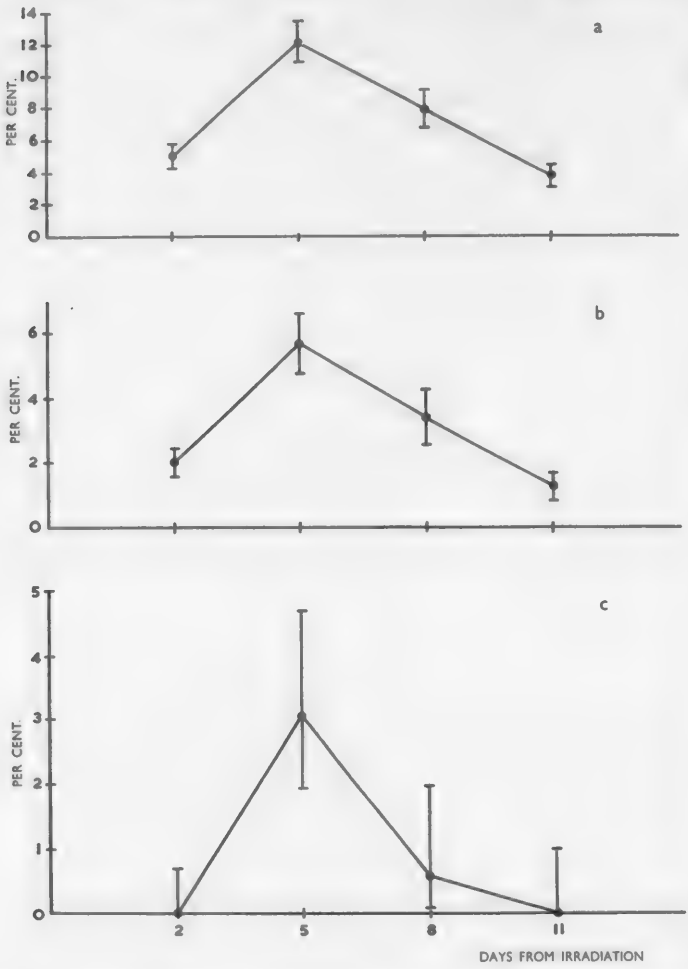

FIG. 2.—Variation in the rate of (*a*) Recessive autosomal lethals (*b*) Recessive sex-linked lethals and (*c*) Translocations on days 2, 5, 8 and 11 following 1000r X-rays.

Standard errors are represented by vertical lines.

1955) been attributed to germinal selection eliminating spermatogonia hemizygous for lethals. The dose similarity, however, between the results obtained for autosomal and sex-linked recessive lethals on all the sampling days, and emphasised by the ratio of mutation rates shown

in the bottom row of table 3, suggests that germinal selection cannot be the main factor. This must be reduced sensitivity of spermatogonia to the induction of mutations. There is, however, a suggestion that the ratio of autosomal to sex-linked lethals is higher on day 11 than previously, so *some* germinal selection may be operative. It is also possible that germinal selection would have been more marked in matings later than day 11. It is clear, however, that the main cause of reduction in yield of mutations from day 5 to day 11 and also the less spectacular reduction from day 2 to day 11 is due to variation in sensitivity to the mutagen, affecting autosomal and sex-linked lethals to similar extents.

(v) *Translocations*

Table 4 and fig. 2*c* show the translocations recovered on the four sampling days following 1000r. Although very few translocations

TABLE 4

Percentage translocations following 1000r *X-rays to males*

Day		2	5	8	11
Total		303	262	171	193
Translocations		0	8	1	2
Per cent. translocations		0·0	3·1	0·6	0·0
SE	upper limit	0·7	4·7	2·0	1·0
	lower limit	0·0	2·0	0·1	0·0

were recovered the results indicate a greater sensitivity for day 5 than any other day. These data are, therefore, consistent with the recessive lethal data rather than with the other structural changes (dominant lethals and deleted X's).

(vi) *Induced crossing-over*

Estimates of the rate of induced crossing-over were made over days 2-13. The results obtained are set out in table 5 and fig. 1*c* and fig. 3. The intention was to provide a means of identifying the specific stages of germ cell development at irradiation corresponding to each day's mating. As crossing-over can only occur in diploid cells, any cross-overs induced will indicate spermatogonia or spermatocytes at irradiation.

The map distances based on crossing-over in the female are *b-pr* 6·0 units, *pr-vg* 12·5 units. In the analysis of cross-over data using three markers, Auerbach (1954) has distinguished two cross-over classes: animals showing a single recessive marker (r_1) and those showing two recessives (r_2). Each r_2 phenotype has a complementary r_1 phenotype which should be produced by crossing-over with

TABLE 5*a*

Frequency of crossing-over and "mutation" in daily samples from males given 1000r X-rays

Day	2	3	4	5	6	7	8	9	10	11	12	13
Non C-O	4240	4675	4256	4576	4616	3895	2919	3966	4713	4876	4293	3717
r_1	0	3	1	1	8	10	9	7	9	5	12	2
r_2	0	0	0	0	1	3	11	6	9	5	12	3
Per cent. C-O	0·00	0·00	0·00	0·00	0·04	0·16	0·75	0·30	0·38	0·20	0·56	0·16
Per cent. "mutation"	0·00	0·06	0·02	0·02	0·15	0·18	−0·07	0·03	0·00	0·00	0·00	−0·03

TABLE 5*b*

Overall frequency of crossing-over and "mutation" for the three periods—days 2-5, days 6-7, days 8-13

	2-5	6-7	8-13
Non C-O	17,747	8,511	24,484
r_1	5	18	44
r_2	0	4	46
$2r_2$	0	8	92
$r_1 - r_2$	5	14	−2
Per cent. C-O	0·00	0·094	0·374
Per cent. "mutation"	0·028	0·164	−0·008

equal frequency. When, therefore, there is an excess of r_1 over r_2 one must suspect causes other than true crossing-over. Apart from crossing-over, r_2 can only be produced by back-mutation at the third locus or deletions large enough to include two loci. On the other hand, r_1 can be produced not only by mutation at that locus (including lethal mutations) but also by small deletions. It follows that r_2 can be taken as a much more reliable estimate of crossing-over than r_1. In the table, therefore, the " cross-overs " have been used to estimate two parameters: $2r_2$ or the true cross-overs, and (r_1-r_2), the mutations and deletions collectively referred to as " mutations ". The results of this analysis are shown in fig. 3. For comparison with the other data, fig. 1*c* shows the values on days 2, 5, 8 and 11 only.

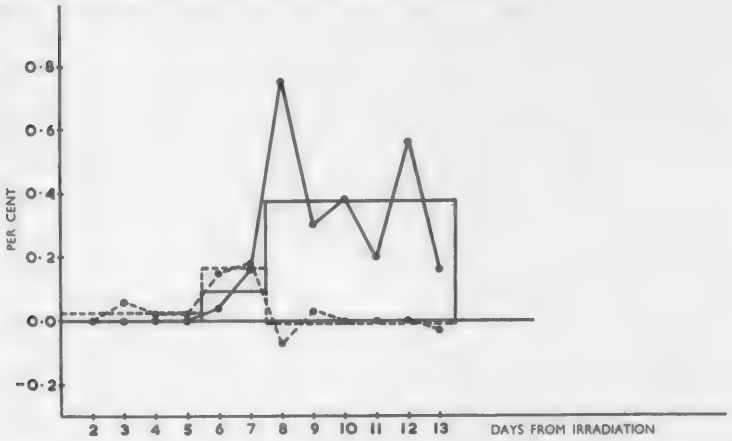

FIG. 3.—Variation in the rate of cross-overs and " mutations " in daily samples following 1000r X-rays. Continuous line equals per cent. cross-overs ($2r_2$). Broken line equals per cent. " mutations " (r_1-r_2). Histograms show the mean level for cross-overs (continuous line) and " mutations " (broken line) over the three periods, days 2-5, 6-7 and 8-13.

The whole period surveyed can be divided into three qualitatively different stages: days 2-5 showing no crossing-over and a low level of " mutations "; days 6 and 7 showing a low level of crossing-over but the highest rate of " mutations " encountered, and days 8-13 during which crossing-over rises to a maximum and deletions are not evident.

Assuming that crossing-over can only occur in diploid cells it seems that days 2-5 represent irradiated sperm and spermatids, days 6-7 spermatocytes, and days 8-13 spermatogonia. The high sensitivity of spermatogonia to the induction of crossing-over is well established (Friesen, 1937, Auerbach, 1954, Sobels and van Steenis, 1957).

The reaction of spermatocytes as such does not appear to have been previously recorded. This stage, in which crossing-over is induced much less readily than in spermatogonia seems particularly susceptible, however, to the induction of small deletions.

Further evidence that many of the r_1 class are not cross-overs is shown by a more detailed analysis. By crossing-over in heterozygotes for *b pr vg*, *pr* animals must be double cross-overs. These would be an extremely rare class, considering the infrequency of even single cross-overs. In all, there were 10 *pr* flies and none of the reciprocal class *b vg*. They occurred in spermatids, spermatocytes and spermatogonia and must represent either mutations or deletions. Out of the total of 100 genuine cross-overs ($2r_2$), 90 occurred in the segment *pr-vg* and only 10 in the segment *b-pr*. Of the putative deletions (r_1-r_2) the loci *b* and *pr* had 10 each, but *vg* had none (estimated as -3).

In fig. 3 there is a suggestion of alternately high and low values for crossing over from day 8 onwards. A χ^2 test for heterogeneity on this part of the data, gave a value of 21·40 for 5 degrees of freedom, which has a probability of 0·001. The biggest contribution to this was 7·99 for day 8, which may represent a special category of germ cell, either just before or just after the last spermatogonial mitosis. In view of the fact that this is the day showing the greatest reduction in fertility after irradiation, there might be one cell stage which is completely eliminated and the mutations observed are from stages on either or both sides of it. In the latter case stages with contrasting sensitivities might be occurring in the same day's sample. Or again, the sperm tested may be from the few survivors of the stage which is most readily sterilised by X-rays. By analogy with the mouse (Oakberg, 1955) this would be the last generation of spermatogonia.

On removing day 8 there is still considerable heterogeneity ($\chi^2_{(4)}=13·98$) with a probability of 0·01. Perhaps this reflects the variation in sensitivity during each successive mitotic cycle.

4. DISCUSSION

The attempt to find other categories of mutation which would repeat the sensitivity pattern of either dominant lethals or deleted X's has evidently failed. On the other hand, sex-linked lethals, autosomal lethals and translocations show closely similar sensitivity patterns. This is especially so if one assumes that translocations, being proportional to a power of the dose greater than unity, would increase more rapidly than recessive lethals with increasing sensitivity of the cell stage.

The sensitivity pattern for dominant lethals up to day 8 could be reconciled with that for these other mutations if one assumed that a high proportion of unhatched eggs from day 8 matings were in fact unfertilised eggs owing to acute shortage of sperm. There is certainly a much lower rate of egg laying on that day (see Bateman, 1956) in a stock where virgin females lay only an occasional egg, so one can assume there is in fact a shortage of sperm. It remains to be established that the egg laying rate is imperfectly adjusted to the amount of sperm available so that when sperm is scarce the fertilisation rate is lowered—

or alternatively that egg laying is proportional to the total sperm, which on day 8 includes a high proportion lacking the capacity for fertilisation.*

In comparing the sensitivity patterns for various types of mutation, allowance must be made for the fact that chromosome rearrangements of whatever kind are never recovered from irradiated spermatogonia. This applies to dominant lethals (*e.g.* Bateman, 1956), deleted X's (Bateman, 1957), translocations and inversions (Ward and Alexander, 1957). In the case of translocations and inversions which are fully viable this cannot be due to the elimination of spermatogonia with cell lethals, though such an explanation could apply to dominant lethals. Either the primary incidence of chromosome breakages in spermatogonia is low, or, with a normal incidence of breakages, *rearrangements* are inhibited, so that all breaks that fail to restitute lead to dominant lethals, which behave as cell lethals in spermatogonia.

Some rearrangements have been observed in *Microtus* spermatogonia by Ockey (1959) following TEM treatment but they were at a very low incidence and all those identified were confined to the sex chromosomes, which are a special case being overwhelmingly heterochromatic.

It follows that in comparisons of sensitivity patterns between rearrangements and mutations, differences for day 11 can be taken for granted. It is the differences over days 2, 5 and 8 which should receive our attention. Over this period, recessive lethals, both autosomal and sex-linked, and translocations show the same pattern. There is a sharp rise from day 2 (sperm) to day 5 (spermatids) followed by a fall on day 8 (spermatocyte or late spermatogonia?) to an intermediate sensitivity. Deleted X's are exceptional with a very different pattern. The rise from day 2 to day 5 is followed by a rise to day 8 which is proportionately the same but in absolute terms is much greater.

What is there special about deleted X's which separates them from the rest? It cannot be that they involve the sex chromosomes (c.f. sex-linked lethals), nor that they are structural changes (c.f. translocations). They have two distinct features, they are *intra*-changes and they are deletions.

In the Drosophila spermatocyte the nucleus is very large in comparison with the amount of chromatin it contains. The bivalents will be widely separated as can be confirmed cytologically in stages where the chromosomes are visible. This could explain why any rearrangements would tend to be intra-changes. Inversions have not been scored in this material. Deletions from the autosomes are lethal when large and when small are not readily detected. However, our data on induced crossing-over are valuable here. The excess of r_1 over r_2 was confined to days 6 and 7, that is to spermatocytes, and it has already been argued that this is probably due to small deletions. We therefore propose that deletions are a mutagenic response more readily induced in spermatocytes than elsewhere. The unique sensitivity pattern of

* *Note added in proof.* We have now verified that in spite of the shortage of sperm, the fertilisation rate on day 8 is normal: thus the high dominant lethal rate is *genuine*.

deleted X's is therefore explained as follows. The variation in post-meiotic germ cells is governed by the general variation in sensitivity to mutagens affecting all kinds of mutational response. Spermatocytes, however, show a special propensity to induced deletions. Owing to the special condition of this type of cell, the most obvious hypothesis is that these deletions are produced by non-homologous crossing-over either between chromosomes or between sister chromatids.

There is one difficulty remaining. The peak incidence of deleted X's is on day 8, whereas the small deletions in our cross-over data were only detected on days 6 and 7. On day 8 however, there was an exceptionally high yield of true cross-overs (r_2) which by error variation could mask the presence of small deletions ($r_1 - r_2$).

5. SUMMARY

F_1 males irradiated with 1000r X-rays were mated on days 2, 5, 8 and 11 after treatment to different classes of female. The incidence of dominant lethals, hyperploids and translocations (structural changes), and recessive autosomal and sex-linked lethals (gene changes) was measured for each day.

Estimates of induced crossing-over in the *b pr vg* region, made in daily samples from day 2 to day 13, were used to identify specific stages of germ cell development. In addition, the excess of animals showing a single recessive marker over those showing two markers was used to estimate the proportion of " mutations " or small deletions.

The pattern of sensitivity was similar for recessive autosomal and sex-linked lethals and translocations, showing a rise from day 2 (sperm) to day 5 (spermatids) followed by a fall on day 8 (spermatocytes or later spermatogonia?).

Deleted X's showed a unique sensitivity pattern with a peak on day 8. This was attributed to the high sensitivity of spermatocytes to intrachanges and in particular to deletions.

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A SYSTEM OF CYTOPLASMIC VARIATION IN *ASPERGILLUS NIDULANS*

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1. INTRODUCTION

ERRATIC variation in the production of perithecia by the homothallic Ascomycete *Aspergillus nidulans* (Eidam), Winter, has been observed in both mycological (Smith, 1957) and genetical studies (Mahoney and Wilkie, 1958). These general observations have shown that the production of perithecia is uncertain and spasmodic. Some strains are regularly sexual while others produce only conidia. Still others give the imperfect form alone through a series of cultures and then suddenly produce abundant perithecia.

Irregular variation in perithecium production might well be of selective advantage in such a homothallic fungus provided the variation is under genotypic control. In this way the organism may combine the ability to exploit asexuality with the versatility of sexual reproduction. This situation could, of course, depend upon a system of forward and back mutation. Indeed, in fungi, where the distinction between somatic and germinal tissue does not exist, a large number of nuclei are potentially capable of providing inocula differing from the parent material. Mitotic recombination and segregation (Pontecorvo, 1956) might also be important in determining irregular variation. An alternative, and perhaps more plausible explanation depends upon the fact that the cytoplasm of the Ascomycetes plays an important part in determining phenotype (Jinks, 1956, 1958). This is all the more plausible since Jinks has found that it is possible to eliminate the sexual stage in *Aspergillus glaucus* by adjustment of the cytoplasm.

Experiments were, therefore, undertaken to determine the nature and control of variation in the sexual phenotype of *A. nidulans*. Three homokaryotic lines of the fungus were used in these investigations. These lines were originally isolated from the wild but had been maintained in the laboratory for at least four years by mass hyphal transfer at 25° C. The methods of propagation and media used are standard for this organism (Jinks, 1952; Pontecorvo *et al.*, 1953). A line marked by the genes *paba1 y; co* (Käffer, 1958), was also employed in the course of the investigations; this was very kindly supplied by Professor G. Pontecorvo.

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2. RESULTS

(i) *Origin of variation*

Mahoney and Wilkie (1958) observed a segregation into perithecial and non-perithecial offspring from wild-type colonies, and the frequency of non-perithecial types was higher at 37° C. than at 25° C.

TABLE 1

Mean number of perithecia in 3 wild lines, scored as total number of perithecia in 5 random fields per colony

Line	Mean at 37° C.	Mean at 25° C.
1	3·96±1·71	1·56±1·10
2	2·20±0·84	4·00±1·70
3	4·52±1·33	0·84±0·54

In no instance, during a number of studies at 25° C. in this laboratory, was any phenotypic variation of such magnitude observed. This applied in the three stock lines which were probably "run down" cytoplasmically by virtue of the continued hyphal propagation. A

TABLE 2

Mean and standard deviation of the number of perithecia in replicate selections for high and low density of perithecia at 37° C. in line 2. Each mean is based on 5 random fields for 5 colonies from each of duplicate plates

Selection		Generations											
		Parent		I		II		III		4		5	
		Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.	Mean	S.D.
Low	1	2·2	0·8	1·2	1·1	6·3	1·8	3·4	2·3	7·7	1·2	9·4	1·3
	2			1·2	0·9	6·6	1·7	7·1	2·7	9·1	2·0	10·2	1·9
High	3			3·2	3·5	9·2	2·6	5·7	1·9	16·8	0·3	18·1	1·9
	4			3·2	4·0	7·3	2·6	10·4	1·0	17·8	1·9	18·2	2·8

comparison was made, therefore, between the conidial offspring of the apparently stable lines at both 37° C. and 25° C. The variation observed in the density of perithecia on each colony was quite large but in none of these colonies were perithecia absent (table 1).

An attempt was then made to establish wholly asexual lines by selection of the least sexual colonies derived from a sample of conidia. Line 2 proved most satisfactory for this purpose at 37° C. Conidia were taken from the two colonies with the lowest density of perithecia and also from the two colonies with the highest density of perithecia.

The response to applying such selection at a pressure of 10 per cent. over five generations is shown in table 2 and fig. 1. From these it can be seen that while the response of the high lines was marked, that for the low lines was not even in the direction of selection. Evidently the cytoplasm varies as it was possible to increase the variation in perithecial density. But, it was not possible to establish asexuality in the way used by Jinks (1956) in *A. glaucus*.

A heterokaryon test to establish the nuclear or cytoplasmic nature of the change in perithecial density was not undertaken but, by

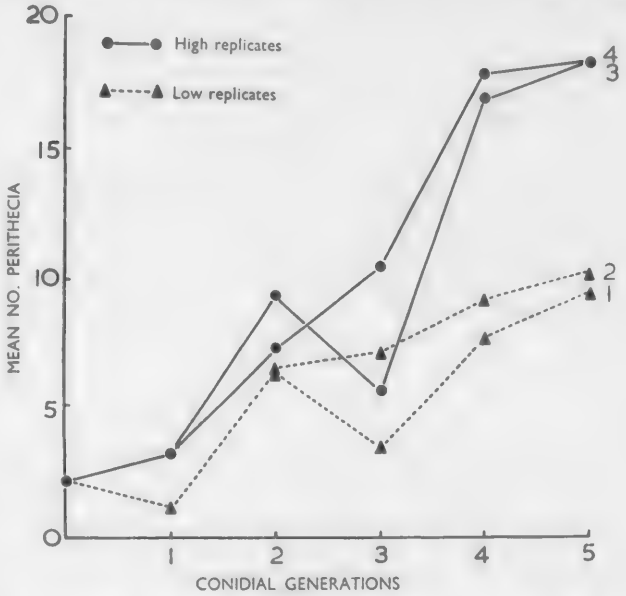

FIG. 1.—The progress of selection; mean number of perithecia in replicate high (3 and 4) and low (1 and 2) selections for density of perithecia. Scored as mean number of perithecia in 5 random fields from 10 colonies.

analogy with Jink's work, the latter is regarded as more probable. This assumption was supported by the behaviour of ascospores from the high and low lines. Determinations of the perithecial density of colonies grown from these ascospores were uniform, as might be expected if the difference between high and low lines was cytoplasmic. This behaviour may be contrasted with the result of the ascospore propagation of a nuclear determined difference (see Section iii). The results of this experiment indicate that, in respect of perithecial density, conidia cannot always maintain the cytoplasm in the state to which it has been reduced by hyphal propagation.

The significance of this result will be discussed later.

(ii) *Morphological variants*

Each stage of selection represented a test of the conidial progeny of wild-type colonies which exhibited differences in the density of perithecia. When the second generation of selection was reached some of the colonies grown from samples of conidia gave morphological variants amongst the apparently wild-type colonies, these variants will be referred to as A to D respectively. A description of the variants and their frequency is given in table 3. Similar variants appeared in all the later stages of the selection experiment (table 4) and were always excluded from the selection itself.

TABLE 3

The morphological variants amongst the conidial offspring, grown at 37° C. and 25° C., from colonies with the highest and lowest mean number of perithecia at 37° C.

Parent		Conidial offspring			
		At 37° C.		At 25° C.	
		Total colonies recovered	Non wild-type	Total colonies recovered	Non wild-type
Low	1	15	2A	6	0
	2	87	1A, 6B	55	■
High	3	63	1A, 2C	65	■
	4	38	1A, 1D	28	■

A-type colonies—a mosaic in appearance including areas of growth covered almost entirely with perithecia or, alternatively, with conidial heads. These colonies also exhibited a high density of sterile hyphae giving an overall woolly-white appearance. B-type colonies—few perithecia and many sterile hyphæ. C-type colonies—very small and with a very high density of perithecia. D-type colonies—wild type in appearance with the additional feature of a brown ring of pigment in the medium around the margin of the colony.

In generation four of the selection experiment several representatives of all four variants were produced at 37° C. Samples of conidia were taken from one A, three B, one C and one D colony at this temperature and grown on minimal medium at both 37° C. and 25° C. The phenotypes of the resulting colonies is given in table 5. With the exception of that from A, the progenies from all the variants showed segregation. The A variant which produced only wild-type offspring was itself seen as one of the products of the B variant.

These variants initially arose only at 37° C. and would easily have been observed had they arisen at 25° C., indeed, when subsequently they did arise at this temperature no difficulty was experienced in distinguishing them. The absence of the variants in the first place at 25° C. can only indicate that their origin was dependent upon temperature. Segregation amongst the progenies even after removal to 25° C.

shows that the change persists long enough to have an effect during conidium production at 25° C.

The segregation of the B and C variants was investigated in more detail:

(*a*) *Segregation of the B variant.* In two instances out of the three examined, the conidial progeny from the B variants was composed of

TABLE 4

The distribution of variants amongst the conidial offspring from 3 generations of sampling wild-type colonies with the highest and lowest mean number of perithecia. Parent wild-type colonies selected at 37° C. Scorings of offspring at 37° C. and 25° C.

Parent		Conidial offspring					
		At 37° C.			At 25° C.		
Sample no.		Total colonies recovered	Wild-type	Variants	Total colonies recovered	Wild-type	Variants
3	Low 1	83	83	0	80	79	1C
	2	18	18	0	24	22	2C
	High 3	270	264	2B, 2C	276	276	0
	4	83	79	3A, 1C	92	89	1A, 1C, 1D
4	Low 1	158	156	2A	115	112	1A, 2D
	2	42	40	2A	67	67	0
	High 3	30	28	2A	24	24	0
	4	252	240	2A, 3B, 1C, 1D	101	100	1D
5	Low 1	172	171	1B	153	152	1B
	2	101	101	0	79	79	0
	High 3	117	117	0	118	117	1B
	4	71	71	0	93	93	0

three distinct classes at both 37° C. and 25° C. The first class consisted of phenotypically wild-type colonies, the second class contained colonies of parental type and the third class was phenotypically indistinguishable from the A variant. Conidia were taken from the wild-type and A type classes at both temperatures and tested at 37° C. and 25° C. The phenotypes of the resulting colonies are given in table 6. The progenies from the A type variants fell into the three classes unlike the initial A progeny tested (table 5), the difference probably depending upon the small sample taken in the latter case. No samples of conidia were taken from B type colonies on the assumption, which subsequently proved to be valid, that they would continue to segregate into three classes. In contrast to the conidial progenies, mass hyphal transfers gave

wild-type growths from all classes. Similarly sexual progenies from the A and B variants in line 2 gave only wild-type progeny.

(*b*) *Segregation of the C variant.* The conidial progeny from the C variant was composed of two classes, at both 37° and 25° C. In one class the colonies were indistinguishable from wild-type, while in the other there were few or no perithecia. The proportions in the two classes were different at the two temperatures. The C variant was itself absent from all but one of these progenies. Conidial samples were taken from both classes and proved to be pure breeding in this and subsequent generations.

TABLE 5

The phenotypes of colonies obtained from conidial samples of A, B, C, and D type variants and grown at 37° C. and 25° C.

Parent		Phenotype of conidial offspring				
		Total colonies recovered	Wild-type	A-type	Parental	Very few or no Perithecia
A	37°	16	16	—	—	—
	25°	11	11	—	—	—
B_1	37°	24	9	[illegible]	7	—
	25°	35	16	7	12	—
B_2	37°	79	24	17	38	—
	25°	108	52	[illegible]	48	—
B_3	37°	72	69	3	—	—
	25°	70	70	—	—	—
D_1	37°	78	43	—	35	—
	25°	206	186	—	20	—
C	37°	75	41	—	—	34
	25°	117	13	1	—	103

TABLE 6

The phenotypes of colonies derived from samples of conidia of apparently wild-type and A-type colonies from the segregation of a B-type variant.

Parent Colony	Phenotype of conidial offspring							
	At 37° C.				At 25° C.			
Phenotype	Total colonies recovered	Wild-type	B-type	A-type	Total colonies recovered	Wild-type	B-type	A-type
From 37° C.								
Wild-type	158	158	0	[illegible]	119	117	[illegible]	[illegible]
A-type 1	61	43	4	14	73	52	2+1D	18
A-type 2	61	59	1	1	55	49	2	4
From 25°C.								
Wild-type	255	255	[illegible]	0	208	207	0	1
A-type 1	138	34	17	87	71	18	33	20
A-type 2	62	13	11	38	61	7	44	10

All the variants obtained during the progress of the selection experiment gave wild-type progeny after mass hyphal transfer. This prevented the application of a heterokaryon test to determine whether the segregating differences were of a nuclear or a cytoplasmic origin. However, even in the absence of such proof, it seems difficult to ascribe the continued segregation of differences through uninucleate conidia to anything except a non-nuclear mechanism. Support for this interpretation follows from the fact that, in a number of cases, segregations of this kind must be attributed to the sorting out of cytoplasmic elements (Arlett, 1957; Jinks, 1959*b*). Clearly, it is often not easy to disentangle the effects of nucleus and cytoplasm (Roper, 1958), in the present case, although proof of the cytoplasmic origin of these variants is not irrefutable, it is regarded as most probable and is presented as such.

TABLE 7

Conidial progeny from 2 heterokaryons between a sterile arginine requiring line and a line requiring p. amino benzoic acid

Heterokaryon	Conidial offspring		
	Total colonies recovered	*paba*1 *y*; *co* fertile	argininеless sterile
1	444	429	15
2	190	188	2

(iii) *Nuclear control of sexuality*

That the A-D variants were cytoplasmic in origin is put beyond any doubt by the sharp contrast when their behaviour, described above, is compared with that of a fifth variant which depends on a stable nuclear change. This variant appeared as a white woolly sector completely devoid of both perithecia and conidia in a stock line requiring argenine. Its rate of growth on medium supplemented with hydrolysed casein was 7·59 mm. per day at 25° C., as compared with 4·35 mm. per day for the strain which gave rise to it. The nuclear nature of the change was established unambiguously by a heterokaryon test. Two heterokaryons were made between the sterile *arginine* line and the line *paba*1 *y*; *co* which required p. amino-benzoic acid for growth. These heterokaryons are, of course, capable of growth on a minimal medium. The classification of their conidial progeny is shown in table 7. It will be seen that the relationship of fertility and sterility to the marker genes was unchanged by passage through the heterokaryon, thus establishing the difference as nuclear.

These progenies also show an excess of the *paba*1 *y*; *co* nuclei in the heterokaryon. A rate of growth comparison showed no differences between *paba*1 *y*; *co* colonies from the heterokaryon and control

colonies which had not been in heterokaryotic association. Hence it was concluded that there was no influence of the sterile *arginine* nuclei on the *paba*1 *y*; *co* nuclei.

There was, however, some effect of association with *paba*1 *y*; *co* on sterile *arginine* progeny. A close examination of the seventeen sterile *arginine* colonies showed that while fourteen were completely sterile three (two from heterokaryon 1 and one from heterokaryon 2) produced a few conidia and perithecia; these numbered less than ten on each colony. Six conidial heads were separately picked off one of these colonies and plated on casein supplemented medium at 25° C. Amongst a total of three hundred and forty-two colonies recovered, two had a few conidial heads but no perithecia, the remaining sister colonies being entirely sterile. Four heads from another of the three colonies gave a total of five hundred and two daughters most of which developed a few conidial heads and perithecia, the number ranging up to 10.

Four perithecia were also crushed from this second colony and the ascospores plated on casein supplemented medium at 25° C. The progeny from each perithecium fell into two classes. Four-fifths were similar to the conidial progeny, both in general appearance and range of variation, while the remainder were completely sterile and, therefore, similar to the original isolate.

Evidently, therefore, the expression of the mutant gene responsible for the sterility in the *arginine* isolate is capable of modification by the cytoplasm for, following association with the cytoplasm of the *paba*1 *y*; *co* strain the degree of sterility varies.

3. DISCUSSION

Two points of significance emerge from these observations. The first is the relation of cytoplasmic variation to temperature. The second is the evidence for cytoplasmic restandardisation in the conidia. The results show that variation is more frequent at 37° C. than at 25° C. That this variation is cytoplasmic in origin is suggested by its failure to pass through ascospores, in contrast to the sterility determined by the nucleus in the *arginine* line. The variation might, of course, be interpreted as resulting from a higher mutation rate at 37° C. than at 25° C. though it seems hardly likely that an increase of such magnitude could be brought about solely by mutation. The more probable explanation is that the increase in variation is due to the behaviour of the cytoplasm being less regular at 37° C. This implies that the cytoplasm is not subject to the precise mechanical control which ensures that nuclear genes reproduce in step with the cell. The regular behaviour at 25° C. must mean, however, that the reproduction of cytoplasmic elements is more or less in step with cell division. Raising the temperature produces the irregularity because the rate of reproduction of the cytoplasm changes. The mechanical and numerical basis of this variation is emphasised in the threshold of the effect which is partly reversible in conidia and totally reversible in the ascospore.

Similar behaviour has also been observed in yeast (Ycas, 1956; James and Spencer, 1958), *Paramecium aurelia* (Preer, 1948) and in tumours induced by *Agrobacterium tumefaciens* (Braun, 1954). In yeast the phenotypes which depend for their origin on an unequal distribution of cytoplasmic particles are more frequent at higher temperatures. Moreover, the multiplication rate of these particles is not wholly dependent upon the rate of division of the cells. The discrepancy is particularly well demonstrated in *P. aurelia* where the frequency of *kappa* cytoplasmic particles varies according to the relative rates of cell division and particle reduplication and may be increased or reduced, at will, by changes in the feeding regime. It is also possible to eliminate the causative principle from tumours induced initially by *A. tumefaciens* by the induction of a high rate of cellular proliferation. Thus disturbances in the equilibrium of cytoplasmic systems would appear to be of widespread occurrence.

The second significant observation follows from the attempt to change the density of perithecia on the fungus by a cytoplasmic selection. Variation does, apparently, occur in the cytoplasm for this character since it was possible to raise the density of perithecia. It was not possible, however, to maintain the density of perithecia at a level to which it had been reduced by hyphal propagation. Hence it would seem that the conidia are incapable of maintaining a cytoplasm of a constitution which regularly exists in hyphæ. Jinks (1956) has shown in *A. glaucus* that a cytoplasm which can pass through conidia cannot pass through ascospores. This is also true of the present stock of *A. nidulans* but, whereas in *A. glaucus* the cytoplasm giving complete sexual sterility will pass through conidia, this is not always true of *A. nidulans*.

Evidently, therefore, conidial propagation in *A. nidulans* has something of the property of the ascospore propagation shown by both species (Mather and Jinks, 1958): it imposes a limit to the range of cytoplasmic variation that can be transmitted. This provides evidence of some, albeit weak, restandardisation of the cytoplasm by the conidia in *A. nidulans*.

The process of restandardisation is seen in a special relation to the nuclear constitution in the sterile *arginine* strain. Initially the mutant gene gave complete sterility but this was modified by association with a mixed cytoplasm, showing how the nucleus and cytoplasm interact in producing the fungal phenotype. Passage through neither the conidium nor ascospore reduces the cytoplasm to complete uniformity in respect of the mutant character. It would seem, therefore, that the process of restandardisation, whether in respect of conidia or ascospores, is related to the wild-type phenotype of the fungus.

The contrast between *A. nidulans* and *A. glaucus* is perhaps of some evolutionary interest. The variation of cytoplasm in hyphæ, conidia and ascospores may be represented as in fig. 2. A wide curve represents the range of cytoplasmic variation in hyphæ, only cytoplasms in the

narrow central portion being transmitted through ascospores. In *A. glaucus* the range of cytoplasm passing the conidia would seem to be similar to that which can exist in the hyphæ but in *A. nidulans* the range appears to be intermediate between hyphæ and ascospores (fig. 2). This is not to say that differences do not exist in *A. glaucus* for it has been shown (Briault, 1956; Jinks, 1959*a*) that, in relation to such systems as adaptation to sugars and poisons, conidia have a narrower range than hyphæ.

The significance can be seen in another way, for *A. glaucus* cannot be propagated indefinitely by conidia or hyphæ alone. The same

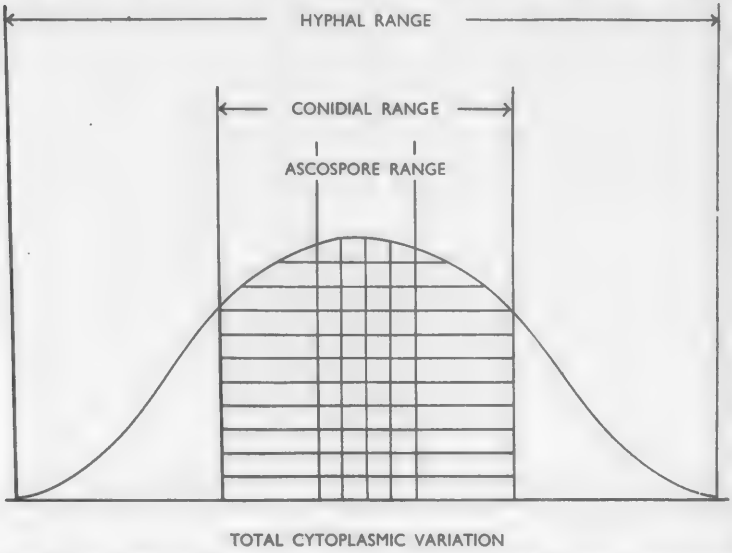

FIG. 2.—The relationship between the mode of propagation and the limits of cytoplasmic variation in *Aspergillus nidulans*.

ageing phenomenon in *A. nidulans*, however, is certainly much slower; and is probably related to a narrower range of variation in the hyphæ and to the effect of the conidia in restoring cytoplasmic balance at least partially. Clearly a fungus like *Aspergillus niger* which is effectively imperfect could not maintain itself if its cytoplasmic balance changed in the way shown by *A. glaucus*. Here only the intervention of sexual reproduction or heterokaryosis will restore the necessary level of balance after continued asexual propagation. Thus, in so far as there is a tendency towards restoration of the cytoplasmic balance in the conidia of *A. nidulans* it may well be that it shows a step towards a feature which is an essential characteristic of an imperfect fungus. This suggests, moreover, that the series *A. glaucus*, *A. nidulans*, *A. niger* is illustrative of the cytoplasmic requirement in the progress to the imperfect fungus.

4. SUMMARY

1. Three lines of *A. nidulans* which had been propagated by hyphæ for four or more years at 25° C. produced morphologically stable conidial offspring at this temperature.

2. Selection amongst the least and most sexual of these wild-type colonies in line 2 at 37° C. showed:

(*a*) The lower limit in sexual phenotype could not be maintained on recourse to conidial propagation, indicating a conidial limit to cytoplasmic variation, and

(*b*) That a range of morphological variants of cytoplasmic origin were induced at a rate of approximately 2 per cent. of the colonies recovered.

3. An instance of nucleo-cytoplasmic interaction in the control of fertility was demonstrated in the behaviour of the conidial progeny from a heterokaryon between a sterile and a morphologically wild-type line.

4. The mechanism for the induction of the cytoplasmic variants is suggested as having arisen out of a possible inequality between the rate of cell division and the rate of division and distribution of cytoplasmic particles.

5. On the evidence of cytoplasmic restandardisation by the conidia of *A. nidulans* it is suggested that the series *A. glaucus*, *A. nidulans*, *A. niger* is illustrative of a cytoplasmic requirement for restandardisation in the progress to the imperfect fungi.

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ASSORTIVE MATING IN A POPULATION IN WHICH TWO ALLELES ARE SEGREGATING

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1. INTRODUCTION

THAT positive assortive mating, the tendency of like to mate with like, occurs in man has long been established. The phenomenon was studied by Fisher (1918). More recently, assortive mating has been found in several polymorphic wild populations. Cooch and Beardmore (1959) found assortive mating in the Blue-Snow Goose complex, and O'Donald (1959) has observed assortive mating among the pale and intermediate and dark phases of the Arctic Skua. In this paper, systems involving assortive mating in populations in which two alleles are segregating will be considered.

2. THE ORIGIN OF ASSORTIVE MATING

The Arctic Skua is of particular interest here as it shows a cline in its phases: the pale phase predominates in the northern part of the range, and dark and intermediate phases predominate in the south. Fisher (1930) has discussed the evolution of such species, where certain alleles are at an advantage at one end of the range, and pass through an intermediate region of diminishing, neutral and negative advantage to the other end of the range. A genetic equilibrium will be established, Fisher suggests " if the increase in frequency in the favourable region and the decrease in frequency in the unfavourable region, not only balance each other quantitatively, but are equal to the rate at which genes diffuse by migration and sexual union, from the one region to the other . . . The longer such an equilibrium is maintained the more numerous will the genetic differences between the types inhabiting extreme regions tend to become, for the situation allows of the extinction of neither the gene favoured locally nor its allelomorph favoured elsewhere, and all new mutations appearing in the intermediate zone which are advantageous at one extreme but disadvantageous at the other will have a chance of being added to the factors in which they differ." So far, there has been no loss of unity in the ancestry of the species. Congenital variations in the means of dispersal will, however, as Fisher points out, lead to the elimination of those individuals that diffuse most, and thus to an increase in genetic isolation. Any tendency among some of the members of the species to assortive mating will still further increase the isolation of the two groups and so diminish the diffusion of the alien gene-complexes in that group where they will be

eliminated. Any genetic incompatibility that arises will further increase this selective effect.

Clines in nature provide a fairly common example of one type of disruptive selection; in general, disruptive selection of a quantitative character controlled at a number of different loci should also give rise, under certain conditions, to assortive mating. Fisher (1930) says of assortive mating in man: " Its principal biometric effects seem to be to increase the genetic variance produced by a given number of Mendelian factors with given gene ratios, and so to increase in a fixed proportion the intensity of the selection to which each is exposed." Thus if disruptive selection is favouring the more extreme deviants in a population while individuals near the mean are at a disadvantage, then those individuals mating with like mates will contribute a greater proportion of descendants to future generations than those mating with dissimilar partners whose offspring, tending to be more heterozygous, will have characters nearer the population mean. Individuals with an inherited preference towards assortive mating will thus increase in the population.

Mather (1955) has argued that stable polymorphism can be the outcome of disruptive selection. This will be so " whenever we can discern a sufficiently strong tie, or co-operation, between structures or entities, whether of genetic structures within the nucleus, of cellular structures within the zygote, or of zygotes themselves within the population, we might expect to find all the features of polymorphism, viz., sharply delimited classes of the structure or entity adjusted to work together for their common advantage, the adjustment depending on a common genotype which can ensure the necessary conditions, and on a switching system whether environmental, segregational or cytoplasmic to direct the structures into one path or the other." Under a system of this kind, it is clear that assortive mating cannot evolve since positive assortive mating implies independence of the optimal phenotypes, one of Mather's conditions for isolation. Similarly in the disruptive selection of a cline, a tie sufficiently strong to inhibit the evolution of assortive mating might result between the alleles, if, under certain conditions, the heterozygote were at an advantage. This system might, however, lead to a balanced equilibrium between the heterozygous advantage and the selection to limit diffusion and thus to a stable polymorphic cline.

3. ASSORTIVE MATING WHEN ONE ALLELE IS DOMINANT

It will be assumed that a proportion α mate with like phenotypes while $1-\alpha$ mate at random. Suppose in a population comprised of the genotypes AA, AB and BB in the proportion u, v and w, A is dominant, so that AA and AB are not recognisably distinct. Let $\alpha(u+v)$ of A phenotypes and αw of B phenotypes mate with like phenotypes while the remainder of the population mates at random. Then among the A phenotypes the tendency to assortive mating will

yield the matings AA×AA, AA×AB and AB×AB in the proportions $\frac{u^2}{(u+v)^2}$, $\frac{2uv}{(u+v)^2}$ and $\frac{v^2}{(u+v)^2}$ out of a total of $a(u+v)$. Thus we shall have

Mating type	*Frequency*
AA×AA	$au^2/(u+v)+(1-a)u^2$
AA×AB	$2auv/(u+v)+2(1-a)uw$
AA×BB	$2(1-a)uw$
AB×AB	$av^2/(u+v)+(1-a)v^2$
AB×BB	$2(1-a)vw$
BB×BB	$aw+(1-a)w^2$

Whence

$$u'=a(u+\tfrac{1}{2}v)^2/(u+v)+(1-a)(u+\tfrac{1}{2}v)^2$$
$$v'=av(u+\tfrac{1}{2}v)/(u+v)+2(1-a)(u+\tfrac{1}{2}v)(w+\tfrac{1}{2}v)$$
$$w'=aw+av^2/4(u+v)+(1-a)(w+\tfrac{1}{2}v)^2$$

where u', v', and w' represent the genotypic proportions in the following generation. If we let p be the frequency of the A allele and q the frequency of B, we have

$$\begin{aligned}p'&=u'+\tfrac{1}{2}v'=(ap^2+avp/2)/(u+v)+(1-a)p\\&=ap(p+\tfrac{1}{2}v)/(p+\tfrac{1}{2}v)+(1-a)p\\&=p.\end{aligned}$$

Thus the gene frequency remains constant. We may therefore put $u=p-\frac{1}{2}v$ and $w=q-\frac{1}{2}v$ to obtain a recurrence relation in the variable v alone:

$$v'=avp/(p+\tfrac{1}{2}v)+2(1-a)pq.$$

The equilibrium position. At equilibrium, when the population is undergoing no further change, we shall have $v'=v$. Thus

$$v=avp/(p+\tfrac{1}{2}v)+2(1-a)pq,$$

which after rearrangement becomes

$$v^2+2p^2(1-a)v-4p^2q(1-a)=0,$$

and

$$v_e=p^2(a-1)\pm p\sqrt{(1-a)[p^2(1-a)+4q]},$$

where v_e denotes the equilibrium value. Clearly for $a<1$ we must have

$$v_e=p^2(a-1)+p\sqrt{(1-a)[p^2(1-a)+4q]},$$

for the alternative root, which we shall call v_e^*, becomes negative. Thus in the case $p=q=\frac{1}{2}$

$$v_e=\tfrac{1}{4}(a-1)+\tfrac{1}{4}\sqrt{(1-a)(9-a)}.$$

Rate of approach to equilibrium. The recurrence relation in v,

$$v'=\alpha vp/(p+\tfrac{1}{2}v)+2(1-\alpha)pq,$$

is non-linear. However, when rewritten in the form

$$v'=\frac{2pv(q+\alpha p)+4(1-\alpha)p^2q}{v+2p},$$

and by letting $v=y/x$,
we have

$$y'/x'=\frac{2py(q+\alpha p)+4(1-\alpha)xp^2q}{y+2px}.$$

Thus

$$\begin{pmatrix}y'\\x'\end{pmatrix}=\begin{pmatrix}2p(q+\alpha p) & 4(1-\alpha)p^2q\\ 1 & 2p\end{pmatrix}\cdot\begin{pmatrix}y\\x\end{pmatrix}$$

and we are required to find the nth power of the matrix.

The characteristic equation is

$$[2p(q+\alpha p)-\lambda][2p-\lambda]-4(1-\alpha)p^2q=0,$$

or

$$\lambda^2-2p\lambda(1+q+\alpha p)+4p^2\alpha=0.$$

Thus

$$\lambda=p(1+q+\alpha p)\pm p\sqrt{(1+q+\alpha p)^2-4\alpha},$$

which after rearrangement becomes

$$\lambda=p(1+q+\alpha p)\pm p\sqrt{(1-\alpha)[p^2(1-\alpha)+4q]}.$$

But

$$p\sqrt{(1-\alpha)[p^2(1-\alpha)+4q]}=v_e-p^2(\alpha-1)$$

and

$$-p\sqrt{(1-\alpha)[p^2(1-\alpha)+4q]}=v_e^*-p^2(\alpha-1).$$

Thus

$$\lambda=v_e+2p \text{ or } v_e^*+2p.$$

To find the principal components of frequency we read the columns of the matrix as homogeneous linear functions of the coefficients a and b (Fisher, 1949). Thus for the latent root v_e+2p we have

$$[2p(q+\alpha p)-v_e-2p].a+b=0$$

or

$$[2p^2(\alpha-1)-v_e].a+b=0.$$

But

$$v_e+v_e^*=2p^2(\alpha-1),$$

and so

$$a=b/(-v_e^*).$$

Similarly, for the latent root $\lambda=v_e^*+2p$,

$$a=b/(-v_e).$$

Thus if the principal components are A and B, we have

$$\begin{pmatrix} A \\ B \end{pmatrix} = \begin{pmatrix} 1 & -v_e^* \\ 1 & -v_e \end{pmatrix} \cdot \begin{pmatrix} y \\ x \end{pmatrix}$$

or

$$\begin{pmatrix} y \\ x \end{pmatrix} = \begin{pmatrix} -v_e & v_e^* \\ -1 & 1 \end{pmatrix} \cdot \begin{pmatrix} A \\ B \end{pmatrix}$$

where we are ignoring the divisor $\begin{vmatrix} 1 & -v_e^* \\ 1 & -v_e \end{vmatrix}$ which will disappear when we put $v=y/x$.

Hence

$$\begin{pmatrix} y_n \\ x_n \end{pmatrix} = \begin{pmatrix} -v_e & v_e^* \\ -1 & 1 \end{pmatrix} \begin{pmatrix} (v_e+2p)^n y_0 - v_e^*(v_e+2p)^n x_0 \\ (v_e^*+2p)^n y_0 - v_e(v_e^*+2p)^n x_0 \end{pmatrix}$$

and so

$$v_n = \frac{v_e^*[(v_e^*+2p)/(v_e+2p)]^n(v_0-v_e) - v_e(v_0-v_e^*)}{[(v_e^*+2p)/(v_e+2p)]^n(v_0-v_e) - (v_0-v_e^*)}.$$

At $n=\infty$ we must have $v_\infty = v_e$, or $(v_e^*+2p)/(v_e+2p) < 1$; that is $v_e^* < v_e$ or $(1-\alpha)[p^2(1-\alpha)+4q] > 0$. Thus the stability condition is simply $\alpha < 1$ which is the case unless mating is completely assortive. It is impossible in the present context to have $\alpha > 1$; in any mathematically equivalent system in which this were possible, it would imply a stable equilibrium at v_e^*.

Mating completely assortive (Case of $\alpha=1$)

When $\alpha=1$, we have $v_e = v_e^* = 0$ and

$$v_n = \frac{v_e^*(v_0-v_e) - v_e(v_0-v_e^*)}{(v_0-v_e) - (v_0-v_e^*)} = 0/0$$

and cannot thus be determined from this equation. If we return to the original matrix, however, which when $\alpha=1$ becomes

$$\begin{pmatrix} 2p & 0 \\ 1 & 2p \end{pmatrix}$$

then direct multiplication suggests

$$\begin{pmatrix} 2p & 0 \\ 1 & 2p \end{pmatrix}^n = \begin{pmatrix} (2p)^n & 0 \\ n(2p)^{n-1} & (2p)^n \end{pmatrix}$$

which is easily proved by induction.

Hence

$$v_n = \frac{(2p)^n v_0}{n(2p)^{n-1} v_0 + (2p)^n},$$

which becomes simply

$$v_n = 2pv_0/(nv_0+2p).$$

Thus in the simplest case of $v_0=\frac{1}{2}$ and $p=\frac{1}{2}$, we find $v_n=1/(n+2)$, giving $v_1=\frac{1}{3}$, $v_2=\frac{1}{4}$, $v_3=\frac{1}{5}$, etc., a result obtained by Jennings (1916).

Table 1 shows the approach to equilibrium for five different values of α when gene frequencies are equal. Wright (1921) has given similar values for $\alpha=0{\cdot}8$ and $0{\cdot}5$, though he does not develop any general formulæ.

TABLE 1

$\alpha=$	0·1	0·3	0·5	0·7	0·9
$n=$ 0	0·5	0·5	0·5	0·5	0·5
1	0·4833	0·4500	0·4167	0·3833	0·35
2	0·4826	0·4431	0·3971	0·3440	0·2833
3	0·4825	0·4421	0·3921	0·3292	0·2487
5	0·4825	0·4420	0·3905	0·3210	0·2178
8	0·4825	0·4419	0·3904	0·3196	0·2042
13	0·4825	0·4419	0·3904	0·3195	0·2004
∞	0·4825	0·4419	0·3904	0·3195	0·2

Thus equilibrium for small values of α lies rather close to that for random mating, and in all cases equilibrium is rapidly attained unless α is almost unity.

4. ASSORTIVE MATING WITHOUT COMPLETE DOMINANCE

The system now to be examined is that in which each genotype has a separate probability of mating with a like genotype. As before, let the genotypic proportions of AA, AB and BB be u, v and w, and let α, β and γ be the respective probabilities of assortive mating. It will then be assumed that the proportion $\mathrm{R}=1-(\alpha u+\beta v+\gamma w)$ of the population mates at random. The frequencies of the mating types will be as follows.

Mating type	*Frequency*
AA × AA	$\alpha u+(1-\alpha)^2u^2/\mathrm{R}$
AA × AB	$2(1-\alpha)(1-\beta)uv/\mathrm{R}$
AA × BB	$2(1-\alpha)(1-\gamma)uw/\mathrm{R}$
AB × AB	$\beta v+(1-\beta)^2v^2/\mathrm{R}$
AB × BB	$2(1-\beta)(1-\gamma)vw/\mathrm{R}$
BB × BB	$\gamma w+(1-\gamma)^2w^2/\mathrm{R}$

Thus if u', v' and w' represent the genotypic proportions in the next generation, we have

$$u'=\alpha u+\tfrac{1}{4}\beta v+[(1-\alpha)u+\tfrac{1}{2}(1-\beta)v]^2/\mathrm{R}$$
$$v'=\tfrac{1}{2}\beta v+2[(1-\alpha)u+\tfrac{1}{2}(1-\beta)v]\times[(1-\gamma)w+\tfrac{1}{2}(1-\beta)v]/\mathrm{R}$$
$$w'=\gamma w+\tfrac{1}{4}\beta v+[(1-\gamma)w+\tfrac{1}{2}(1-\beta)v]^2/\mathrm{R}.$$

It may easily be shown that $u'+\frac{1}{2}v'=u+\frac{1}{2}v$ and hence that the gene frequency remains constant. Thus we may put $u=p-\frac{1}{2}v$ and $w=q-\frac{1}{2}v$,

where p and q are the frequencies of the alleles A and B respectively. Substitution leads, after some reduction, to a recurrence relation in v alone:

$$v'=\tfrac{1}{2}\beta v+\frac{2[(1-\alpha)p+\frac{1}{2}(\alpha-\beta)v][(1-\gamma)q+\frac{1}{2}(\gamma-\beta)v]}{1-(\alpha p+\gamma q)+v(\frac{1}{2}\alpha-\beta+\frac{1}{2}\gamma)}.$$

In the simple case of $\alpha=\beta=\gamma$, the recurrence relation reduces to that for mixed selfing and random mating:

$$v'=\tfrac{1}{2}\alpha v+2pq(1-\alpha).$$

Thus at equilibrium $v_e=2pq(1-\alpha)/(1-\frac{1}{2}\alpha)$. For stability we must have $\frac{1}{2}\alpha<1$ or $\alpha<2$, and the rate of approach to equilibrium will be determined by $-\log_e \frac{1}{2}\alpha$. This model never reduces to that of complete dominance, however, since all genotypes are assumed to be recognisably distinct.

A linear equation may be obtained in the general case if it is assumed that α, β and γ are small, so that quadratic and higher terms in these parameters may be ignored. This gives

$$v'=(2pq\beta-\tfrac{1}{2}\beta+\alpha q^2+\gamma p^2)v+2pq(1-\alpha q-\gamma p),$$

and at equilibrium

$$v_e=2pq(1-\tfrac{1}{2}\beta+2\beta pq-\alpha pq-\gamma pq).$$

For stability $2\beta pq-\frac{1}{2}\beta+\alpha q^2+\gamma p^2<1$; when $p=q$ this condition becomes $\frac{1}{4}\alpha+\frac{1}{4}\gamma<1$. If $\alpha=\gamma$ this reduces to the stability condition found exactly for the equilibrium between mixed selfing and random mating. In general, whenever the parameters α, β and γ are at all small, this solution will be very accurate. Thus when $\alpha=\gamma=\frac{1}{2}$ and $p=q$, $v_e=1/3$ exactly, but 3/8 by the approximate treatment. This exceeds the exact value by 1/24. If, however, $\alpha=\gamma=\frac{1}{4}$ and $p=q$, the approximate value of v_e exceeds the exact value of 3/7 by only 1/112. An exact solution of the general recurrence equation, however, is available as one of the roots of the quadratic

$$[(1-\tfrac{1}{2}\beta)(\tfrac{1}{2}\alpha-\beta+\tfrac{1}{2}\gamma)-\tfrac{1}{2}(\alpha-\beta)(\gamma-\beta)]v^2+[(1-\tfrac{1}{2}\beta)(1-\alpha p-\gamma q)$$
$$-(1-\gamma)(\alpha-\beta)q-(1-\alpha)(\gamma-\beta)p]v-2pq(1-\alpha)(1-\gamma)=0.$$

Thus if $\alpha=0\cdot3$, $\beta=0\cdot2$ and $\gamma=0\cdot1$ and $p=q$, $v_e=0\cdot4423$, whereas the approximate formula gives 0·45. When α, β and γ become very small, however, the exact formula becomes very troublesome owing to the need to extract a square root to many places of decimals. As an example, when $\alpha=0\cdot003$, $\beta=0\cdot002$ and $\gamma=0\cdot001$,

$$v_e=\frac{-1\cdot994002+\sqrt{3\cdot97604796001 6}}{0\cdot000002}.$$

The approximate method gives 0·4995, while to obtain this accuracy from the exact treatment, the above square root has to be extracted to ten significant figures as 1·994002999. In this case $-\log_e(\frac{1}{4}\alpha+\frac{1}{4}\gamma)$ $=6{\cdot}907755$. For complete selfing, with no random mating, the equivalent value is 0·69315. Thus equilibrium is approached 9·966 times as fast as homozygosity is approached in complete selfing.

5. SUMMARY

The object of this paper has been to discuss the origin of assortive mating and its effects on populations in which two alleles are segregating. The calculations given in the last two sections demonstrate that in a population in which a few individuals mate only with like mates, the equilibrium position, which is rapidly approached, lies rather close to that of random mating. Thus in a population that is in the process of splitting into two groups, assortive mating, if only slightly developed, should be a relatively small factor in the genetic isolation of the diverging groups. Darwinian selection, however, can operate only by the differential survival of individuals, and in the relative numbers of offspring they produce. From this point of view it is clear that the evolution of assortive mating will, in general, be rather slow. Only if in some individuals the preference for assortive mating is strongly developed will the selective forces discussed in Section 3 have large effects on which to act.

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THE EXCHANGE OF GENES BETWEEN NUCLEI OF A DIKARYON

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1. INTRODUCTION

THE life cycle of many Basidiomycetes involves a union between compatible monokaryotic strains in which a mutual exchange of nuclei takes place. The process is unusual in that there is a prolonged phase of growth between plasmogamy and karyogamy. The cells of the fruit body and of a variable amount of mycelium do not contain a diploid nucleus but a dikaryon, or pair of haploid nuclei, which divide synchronously, a daughter from each nucleus passing into each new cell. Fusion of the member nuclei of the dikaryon takes place in the basidia, the only cells in which a diploid nucleus has been observed, and is immediately followed by a reduction division and the formation of the haploid basidiospores. It would therefore be expected that genetic recombination would be confined to the nuclear divisions within the basidia. Nevertheless, investigations of mating reactions have yielded results which seem to controvert this conclusion.

The mating system of *Schizophyllum commune*, *Coprinus lagopus* and many other Basidiomycetes is controlled by a series of multiple alleles at each of two unlinked loci, A and B. When monokaryotic (sometimes called homokaryotic) mycelia having different alleles at both the loci, (*e.g.* $A^1.B^1$ and $A^2.B^2$), come into contact, an exchange of nuclei takes place after which both mycelia become dikaryotic and are able to produce the typical fruit bodies. The fusion nucleus, being heterozygous at both loci, can give rise to monokaryons of four different mating types, $A^1.B^1$, $A^1.B^2$, $A^2.B^1$ and $A^2.B^2$. Pairs of mycelia with a common allele exchange nuclei but remain sterile.

The result of mating a monokaryotic mycelium with a dikaryotic mycelium was studied in *Coprinus lagopus* by Buller (1931). Three kinds of mating were described: (i) compatible matings in which the monokaryon was compatible with both nuclei of the dikaryon, *e.g.* $(A^1.B^1+A^2.B^2)\times A^3.B^3$, (ii) semi-compatible (or hemi-compatible) matings in which the monokaryon was compatible with one nucleus of the dikaryon, *e.g.* $(A^1.B^1+A^2.B^2)\times A^1.B^1$ and (iii) incompatible (or non-compatible) matings in which the monokaryon was compatible with neither nucleus of the dikaryon, *e.g.* $(A^1.B^1+A^2.B^2)\times A^1.B^2$. The monokaryotic mycelium became dikaryotic in all compatible and semi-compatible matings. The remarkable fact was that dikaryotisation of the monokaryon also occurred in some of the incompatible matings although the reaction was slow and patchy and often failed

altogether. One explanation suggested was that the dikaryon may be able to produce new mating types which, in these anomalous matings, were compatible with the monokaryons.

Quintanilha (1938) and Papazian (1954) extended Buller's observations. Quintanilha's results leave no doubt that a dikaryon $A^1.B^1 + A^2.B^2$ can produce nuclei which are either $A^1.B^2$ or $A^2.B^1$ outside the basidia, but gave no clue to the genetic mechanism involved in the change. Papazian confirmed previous results and by using two marker genes obtained data from three incompatible matings which indicated that recombination of mating type alleles may have occurred between the nuclei of the dikaryon in the hyphal cells. However, the possibility of mutation could not be ruled out.

In an earlier study of compatible matings between monokaryotic and dikaryotic mycelia by Papazian (1950), the further remarkable fact emerged that these matings, like the incompatible combinations, produced new mating types during the vegetative multiplication of the dikaryon despite the compatibility of the parental genotypes. This unexpected result suggested that the nuclei with non-parental mating types may be present in considerable numbers since in a compatible mating they have no obvious selective advantage.

The following experiments with *Schizophyllum commune* were an attempt to determine how the non-parental genotypes arise during the vegetative multiplication of the dikaryon.

2. MATERIAL AND METHODS

The laboratory stock strains of *Schizophyllum commune* used in these experiments were $A^{41}c.B^{41}$ (347), $A^{41}.B^{41}$ (699), $A^6.B^6f$ (108), $A^9.B^{10}.i$ (130) and $A^3.B^2.u$ (73) (Raper and Miles 1958). From appropriate crosses of these strains other required genotypes were obtained.

TABLE 1

Linkage data and phenotypes of relevant genes in Schizophyllum commune

Linkage group	Locus	Map distance	Effect
1	A *c*	44 units	Incompatibility Concentric ring pattern on mycelium
2	B *f*	32 units	Incompatibility Colonies with fir-like margins
3	*i**	—	Indigo pigment present
4	*u*	—	Requires uracil

* Identical with the " early blue " mutant (Raper and Miles, 1958).

The linkage groups and phenotypes are described in table 1. None of the mutants is expressed when present in only one nucleus of a dikaryon and none has been known to mutate back to the wild type allele.

Matings were made on standard medium (Snider and Raper, 1958) in 15 cm. Petri dishes. The monokaryotic mycelium was inoculated first, followed after 48 hours by inoculation with an overlapping disc of dikaryotic mycelium. After an interval of 7 days, new dikaryotic mycelium was present on the fringe of growth emanating from the monokaryotic inoculum.

An agar block bearing hyphæ of the new dikaryotic mycelium was transferred to fruiting medium and after 5 to 10 days, mature fruit bodies were formed.

One fruit body from each new dikaryotic mycelium was suspended over a fresh plate containing standard medium until a sufficient number of spores were shed. The spore deposit was covered with a few drops of sterile water and spread evenly over the plate with a glass rod.

After 24 hours, 20 single germlings from each fruit body were isolated and transferred to small screw-top bottles containing slants of standard medium. Subsequent growth in bottles showed whether the isolates carried the *fir* (*f*), *concentric* (*c*) or *indigo* (*i*) genes. Where the *uracilless* (*u*) mutant was involved in a cross, a small block of mycelium was removed from the bottles and placed on minimal (*i.e.* fruiting) medium. After a second transfer to minimal medium the growth was observed. *Uracilless* isolates produced no growth while others made rapidly spreading colonies. The suspected *uracilless* mutants were rescued to minimal medium supplemented with uracil to confirm their specific requirement.

Six monokaryotic testers were used in the mating type analysis. Three were so constituted that each A allele present in the original mating was combined with a B allele not present in the mated strains. Incompatibility with these testers could only be due to common A alleles. The B alleles were identified on the same basis.

Dikaryotic mycelia of *S. commune* have widely different capacities to produce fruit bodies. This variation has a genetic basis (Raper and Krongelb, 1958). To avoid problems due to infertility, all strains were crossed to $A^{41}.B^{41}$ (699) for four generations since this is known to be a highly productive strain.

The arrangement of marker genes was basically alike in all matings. The monokaryon was marked with a mutant which was not linked with either the A or the B loci. In the dikaryon, one of the nuclei carried a mutant linked with the A locus while the other included a mutant linked with the B locus. The choice and arrangement of markers was restricted since *fir* (*f*) was the only available marker for the B locus and *concentric* (*c*) the only mutant linked with A which would form a dikaryon in association with a nucleus carrying *fir*.

In describing matings between monokaryotic and dikaryotic strains, the term dimon will be adopted following Papazian's usage. The inoculated dikaryotic mycelium will be referred to as the parental dikaryotic mycelium to distinguish it from the new dikaryotic mycelium which arises as a result of the migration of nuclei into the monokaryotic mate.

The design of the experiments detects recombination only between nuclei of the parental dikaryon and then only when the recombinant types enter into the new dikaryon.

3. RESULTS

(i) Series 1. $A^9.B^{10}.1\times(A^6c.B^{41}+A^{41}.B^6f)$

Forty-eight replicate matings of the above kind were analysed. There were four reactions.

(*a*) In the first class thirty-one replicates produced a new dikaryotic mycelium from which the mating types $A^9.B^{10}$, $A^6.B^{10}$, $A^9.B^{41}$ and $A^6.B^{41}$ segregated in the basidiospore progeny. The mutants *indigo* (*i*) and *concentric* (*c*) also segregated in every family. The alleles A^6, B^{41} and *c* were associated in one nucleus of the parental dikaryon and it is

therefore concluded that this nucleus migrated and formed the new dikaryon with the resident nucleus, $A^9.B^{10}.i$ of the monokaryotic mycelium.

(*b*) In the second class of which there were six representatives, the new dikaryons produced the mating types $A^6.B^{41}$, $A^{41}.B^6$, $A^{41}.B^{41}$ and $A^6.B^6$. The mutants *concentric* (*c*) and *fir* (*f*) segregated in each of the six families. The A and B alleles and the markers are those which were present in the parental dikaryon. There is therefore reason to believe that the nuclei of the dikaryon can migrate simultaneously, maintaining their association despite the presence of a compatible nucleus in the monokaryotic mycelium through which they pass.

(*c*) The third class consisted of six families which can be described under three headings.

In four of the replicates the mating types $A^9.B^{10}$, $A^6.B^6$, $A^9.B^6$ and $A^6.B^{10}$ were present in the progeny. *Concentric* (*c*), the marker linked with A^6, *fir* (*f*), the marker linked with B^6 and *indigo* (*i*) were also present in each of the four families. A^6 and B^6 were not present in the same nucleus of the parental dikaryon. Nevertheless they appeared in the progeny from four newly established dikaryotic mycelia associated with their respective markers while A^{41} and B^{41} were absent. Therefore the new genotype $A^6c.B^6f$ is presumed to have arisen by an exchange of genes between the two nuclei of the parental dikaryon and this recombinant nucleus to have subsequently established the new dikaryon with the nucleus from the monokaryotic parent.

A fifth member of the class was identical in all respects to the four described above except for the absence of *fir* (*f*) segregants in the progeny. Thirty additional single spore isolates were examined none of which turned out to be a *fir* colony. The chance of a sampling error can therefore be dismissed. The origin of this single dikaryotic mycelium is once again attributed to recombination between the nuclei $A^6c.B^{41}$ and $A^{41}.B^6f$ to give a new genotype $A^6c.B^6$. Because of the absence of the *fir* (*f*) marker, this example was the first indication that the exchanges between the nuclei of the parental dikaryon may not necessarily involve whole chromosomes.

The last member of this class produced progeny with the mating types $A^{41}.B^{41}$, $A^{41}.B^{10}$, $A^9.B^{41}$ and $A^9.B^{10}$. The only mutant to segregate was *indigo* (*i*). A^{41} and B^{41} were present in the parental dikaryon but in different nuclei and they were not associated with any markers. In the light of the previous examples and in the absence of A^6, B^6, *concentric* (*c*) and *fir* (*f*) alleles in this family, it seems reasonable to conclude that a new genotype $A^{41}.B^{41}$ arose by recombination and entered into the new dikaryon.

(*d*) The fourth class was a heterogeneous collection but its five constituent members shared the common property of sterility. None of the new dikaryotic mycelia produced fruit bodies. On subculturing, three of the sterile mycelia proved to be unstable. Clamp connections were no longer present, the cultures were *indigo* in colour and in each

case the mating type was $A^9.B^{10}$ thus indicating that these three strains had reverted to a monokaryotic state.

The two remaining sterile dikaryotic mycelia were stable. Because of their sterility, a new approach was necessary to find out their genotype. They were first mated with a completely unrelated monokaryotic strain $A^1.B^2$. The new dikaryons produced as a result of these dimon matings were allowed to fruit and in both cases *indigo* colonies and the alleles A^9 and B^{10} were recovered in the progeny, indicating that $A^9.B^{10}.i$ was one of the nuclei present in the sterile dikaryotic parents. The other nucleus could have carried $A^{41}.B^6$, $A^6.B^{41}$ or a new combination of these alleles. To distinguish between the alternatives, the two sterile dikaryons were mated with $A^{41}.B^{10}$, $A^6.B^{10}$, $A^9.B^6$ and $A^9.B^{41}$ as testers. The results of these matings showed that in one of the dikaryotic mycelia the second nucleus carried the alleles A^6 and B^{41}, the full genotype therefore being $A^9.B^{10}+A^6.B^{41}$. In the other dikaryon the second nucleus had the recombinant genotype $A^6.B^6$. The test matings produced dikaryons which were again sterile and consequently attempts to determine which markers were present were abandoned.

(ii) *Series* 2. $A^9.B^{10}.1\times(A^6B^{41}f+A^{41}c.B^6)$

Ten replicates of the above cross were analysed. The results showed that seven of the newly-formed dikaryons combined the nucleus of the monokaryotic parent with one of the nuclei of the parental dikaryon. These dikaryons had the genotype $A^9.B^{10}.i+A^6.B^{41}f$ and their origin was the same as the first category in the previous series.

In one case the new dikaryon was genetically identical with the parental dikaryon thus corresponding with the second class in the first series of replicates. The last two replicates produced new dikaryotic mycelia which proved to have the genotype $A^9.B^{10}.i+A^6.B^6$. These are assumed to have arisen after the production of the recombinant genotype $A^6.B^6$ in the parental dikaryon and therefore correspond with the third class in the first series.

(iii) *Series* 3. $A^2.B^2.u\times(A^{41}c.B^{41}+A^6.B^6f)$

There were twenty-one replicates of this dimon mating. Again classes (*a*), (*b*) and (*c*) of the first series were represented. In sixteen new dikaryons the nucleus of the monokaryotic parent was associated with one of the nuclei from the parental dikaryon. Of these, ten had the genotype $A^2.B^2.u+A^{41}c.B^{41}$ and six were $A^2.B^2.u+A^6.B^6f$. In one of the new dikaryons the genotype was the same as that of the parental dikaryon. There were three examples of recombination; $A^2.B^2.u+A^{41}.B^6f$, $A^2.B^2.u+A^{41}c.B^6$ and $A^2.B^2.(S)+A^6.B^{41}$.

The last member of this series was unique. The new dikaryon had the genotype $A^2.B^2+A^6.B^6$ and therefore had two non-parental genotypes. One of these is probably a recombinant derived from the parental dikaryon but the origin of the other, which had the mating

type of the parental monokaryon although the marker was absent, is doubtful. It may be that there was a back mutation of the *uracilless* gene to the wild type allele or alternatively there may have been

TABLE 2

A summary of the results of seventy-six dimon matings in Schizophyllum commune

Dikaryon	Migrating nuclei			
	Parental		Recombinant	
	One	Both	Independent assortment	Crossing-over
$A^6c.B^{41}+A^{41}.B^6f$	32	II	6	1
$A^6.B^{41}f.+A^{41}c.B^6$	7	1	2	—
$A^{41}c.B^{41}+A^6.B^6f$	16	1	1	3
Total	55	II	9	4
Percentage	72·3	10·5	11·8	5·1

recombination between the $A^2.B^2.u$ nucleus and another of the parental nuclei which was not included in the new dikaryon.

The results of the three series of matings are summarised in table 2.

4. DISCUSSION

Following Buller's demonstration of the unexpected dikaryotisation of the monokaryotic parent in incompatible dimon matings, three different interpretations were proposed.

(i) *Migration of the dikaryotic nuclei*

Buller himself suggested that the nuclei of the dikaryon migrated and supplanted the resident nucleus in the monokaryotic mycelium. This interpretation was also supported by Dickson (1935), Chow (1934) and Oikawa (1939). From the data presented in the present paper, it would seem that migration of the dikaryotic nuclei does occur in a minority of matings. Although there is no proof that in these cases an occasional hypha of the parental dikaryon does not penetrate the interstices of the monokaryotic mycelium, Terekawa's (1957) observations of nuclei migrating from a dikaryon in pairs lend support to the interpretation of synchronous migration of the dikaryon's nuclei.

(ii) *Mutation*

The second proposal involved the mutation of one of the incompatibility genes to an allele which rendered one of the nuclei of the dikaryon compatible with the monokaryon. As evidence from dimon

matings accumulated, this explanation became increasingly improbable since the mating type of the new nucleus always included the A allele of one nucleus of the dikaryon and the B allele of the other. The use of markers has now shown that in certain cases two mutations would be required and this is doubly unlikely. There is indeed no evidence which supports this explanation in preference to others.

(iii) *Recombination*

Quintanilha and Papazian considered that new nuclei arose from elements of the existing dikaryon. Without mutants which are linked with the A and B loci it is impossible to discriminate between mutation and recombination of these loci and neither author had the necessary mutants available. In *S. commune* however, markers became available and their use in the present experiments vindicates Quintanilha's and Papazian's conclusions.

The validity of the recombination theory in relation to dimon matings rests of course, on the premise that the fruit body of *S. commune* is never composed of sectors in which the cells contain genetically different nuclei. The experimental evidence in favour of the genetic homogeneity of the fruit body is, however, sound. In the course of investigations by a number of different workers, the progeny from a very large number of fruit bodies of *S. commune* have been analysed and a chimæra has never been reported. So there can be little doubt that the non-parental genotypes recovered in these experiments are the products of genuine recombination occurring during the multiplication of the nuclei in the parental dikaryotic mycelium.

Some of the recombinant genotypes appear to have been the result of independent assortment of whole chromosomes while others can only have arisen by crossing-over between homologous chromosomes. Although the mechanics by which these events occur are far from clear, crossing-over requires pairing of chromosomes and this can take place only in a fusion nucleus. The behaviour of *S. commune* therefore reveals an apparent contradiction. Cytological observations indicate that the only diploid nuclei are in the basidia. Genetical results indicate that diploidy must also occur in other cells. However there are so far no data on which to predict accurately the frequency of these diploid nuclei and it may be that their incidence is rare enough to account for the discrepancy.

The fusion of the nuclei of the dikaryon in hyphal cells poses the question of how the nuclei are again reduced to the haploid state. A prolonged diploid phase is unlikely in view of the cytological data. Since diploidy is the immediate prelude to reduction in the basidia it may also be so in the hyphal cells. Again it is not known whether the recombination is the result of meiosis or a parasexual mechanism such as that demonstrated in a number of Ascomycetes (Pontecorvo and Kafer, 1958). This latter interesting possibility must be taken into account in future investigations.

In conclusion, the significant deviation from an expected 1 : 1 ratio in the occurrence of $A^9.B^{10}.i+A^{41}.B^6f$ and $A^9.B^{10}.i+A^6c.B^{41}$ dikaryons in the first series of replicate matings and a comparable deviation in the second series is not at present understood. Such deviations are by no means uncommon. Kimura (1954) studied this effect in *Coprinus microrhizus* to discover what factors decide the choice of dikaryotising nuclei in compatible dimon matings. He concluded that a cytoplasmic factor was involved. Preliminary tests with *S. commune* suggest that the choice of dikaryotising nuclei is directly influenced by the mating types involved and more particularly by the structure of the A and B alleles as described by Raper, Baxter and Middleton (1958). The problem of differential affinity between mating types is the subject of further experiments.

5. SUMMARY

The dikaryotisation of monokaryons in compatible dimon matings of *S. commune* occurs in two ways.

1. By the association of the nucleus of the monokaryon with one of the parental nuclei of the dikaryon.

2. By the association of the nucleus of the monokaryon with a nucleus of non-parental genotype from the dikaryon.

In addition, both nuclei of the dikaryon may migrate intact through the monokaryotic mycelium.

Using genetic markers, it has been shown that non-parental mating types arise by recombination between the two component nuclei of the dikaryon in the absence of fruit bodies and basidiospores. In some cases the exchange of genetic material seems to result from independent assortment of chromosomes and in others, from crossing-over between homologous chromosomes.

It is uncertain whether recombination is caused by the parasexual processes of haploidisation and mitotic recombination such as have been described in Ascomycetes or by precocious fusion of the nuclei of the dikaryon followed by meiosis.

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THE DIPLOIDISATION OF POLYPLOID WHEAT

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1. INTRODUCTION

Allopolyploids are usually considered to have originated from species-hybrids in which there was little or no meiotic chromosome pairing because of the structural dissimilarities of the chromosome sets of the parental species. The same structural distinctions are also held to be responsible for the absence of multivalents in the derived polyploids, since they would limit heterogenetic associations (Waddington, 1950) between chromosomes of the different basic sets. Indeed, it has been thought that the differential affinity which causes each chromosome to pair with its identical partner may often be so great that only bivalents are formed.

However, an examination of many of the frequently quoted examples of allopolyploid behaviour suggests that the direct origin of polyploids free from heterogenetic multivalents, with their depressing influence on fertility and stability, must be rare. Indeed, the cytologically diploid behaviour which many natural polyploid species display must often have been acquired subsequent to the development of polyploidy.

Hitherto there has been no evidence to show how the cytological diploidisation of a natural polyploid has been achieved. However, recent experimental results with the hexaploid common wheat, *Triticum æstivum*, illustrate the basis of diploidisation in an apparently typical allopolyploid species, and at the same time give an insight into the cytogenetic functioning of a polyploid (Riley and Chapman, 1958*b*). The purpose of the present paper is to collect, extend and discuss the evidence so far available on the diploidisation of wheat.

2. THE STRUCTURE OF *TRITICUM ÆSTIVUM*

It may be helpful, at the outset, to outline the cytogenetic composition of *Triticum æstivum*, and so provide a background for the discussion of the meiotic organisation of the species. *T. æstivum* is the name currently applied to the common wheat of agriculture, for long referred to as *T. vulgare*. The taxonomic validity of these names has been discussed by Bowden (1959).

The polyploid series in the genus *Triticum* includes diploid, tetraploid and hexaploid species with 14, 28 and 42 chromosomes respectively. The haploid set of chromosomes, or genome, of the diploid wheats may be given the symbol A, so that the diploids are genomically AA. The full complement of diploid wheat is also found in the tetraploid

and hexaploid species (Sears, 1948). In most, and possibly all, of the tetraploids the complete set of chromosomes of another diploid species, most probably *Ægilops speltoides*, is combined with the A genome (Riley, Unrau and Chapman, 1958). The second genome is given the symbol B, so that the tetraploid wheats have the constitution AABB.

Hexaploid wheats have the full complement of chromosomes of the tetraploids and to this has been added the full D set of the diploid *Ægilops squarrosa*. Consequently the hexaploid wheats, including *T. æstivum*, have the genome constitution AABBDD.

The relationships between the chromosome sets of the wheats and their relatives have been mainly established by the study of meiotic pairing in species hybrids. The presence of a common genome was revealed by the close synapsis of chromosomes of the related sets. That genome analysis has been possible, in this way, must imply that no structural alterations, of sufficient magnitude seriously to interrupt pairing, have occurred since the separation of the sets of chromosomes common to both parents. Thus the genomes of the tetraploid and hexaploid species are in essentially the same state as those of the original diploids from which they were derived.

In hybrids made between the diploid species, which have been involved in the parentage of *T. æstivum*, considerable chromosome pairing takes place. Indeed, there is occasionally full pairing to give seven bivalents in the AD hybrid between diploid wheat and *A. squarrosa* and in what is probably the AB hybrid, between diploid wheat and *A. speltoides*, although the mean frequency for both hybrids is between 3 and 4 bivalents per cell (Sears, 1941; Riley, Unrau and Chapman, 1958). Moreover, the attraction between the chromosomes of these parental diploids is so great that, in the synthetic tetraploids derived from the diploid hybrids, heterogenetic multivalents are common (Sears, 1941; Riley, Unrau and Chapman, 1958).

However, although their chromosomes are structurally similar, and are still essentially unaltered compared with their condition in the diploids, there is no pairing between the different genomes in hexaploid wheat. Indeed, even in 21-chromosome haploids of wheat, in which there is no differential affinity to restrict pairing to fully homologous partners, very little heterogenetic synapsis takes place. Consequently the affinity between equivalent chromosomes of the different genomes must have been suppressed or removed.

This anomaly is emphasised by the extremely close genetical relationships between corresponding chromosomes of different genomes, which have been demonstrated by nullisomic-tetrasomic compensation (Sears, 1954). This technique involves crossing together 41-chromosome monosomics and 44-chromosome tetrasomics in which the chromosomes respectively monosomic and tetrasomic are not homologous. Amongst the derivatives of the resulting hybrids it is possible to extract 42-chromosome plants nullisomic for the chromosome which

was originally monosomic in one parent, but still tetrasomic for the chromosome originally tetrasomic in the other parent. Where the nullisomic and tetrasomic chromosomes have similar genetic properties, the physiological defects caused by the complete absence of one chromosome are made up by the extra dosage of the other. Such compensation does not occur when the genetic activities of the pairs involved are not alike.

From this work it has been possible to show that the chromosome complement of wheat can be arranged into seven groups each of three pairs (Sears, 1954). Within the groups nullisomic-tetrasomic compensation occurs and there is some equivalence of genetic activity, whereas between groups there is little similarity in activity. Following Huskins (1931) the term "homœologous" meaning "similar" has been applied to the relationship between non-partner chromosomes in the same group. The term "homologous", meaning the "same", has been retained to describe the relationship between partner chromosomes.

Recently each homœologous group has been shown to have one chromosome pair in each of the three genomes (Sears, 1958). Thus the relationships which have been established presumably imply the recognition, in the genomes of the hexaploid, of corresponding chromosomes from the three original diploid parents.

Thus, the chromosomes of the original diploid parents of wheat are sufficiently similar to be capable of allosyndetic conjugation in hybrids. Moreover they have undergone no major structural modifications since their incorporation in the polyploids. In addition the similarity in genetic activity of corresponding chromosomes is retained. Yet thereis no meiotic pairing between homœologous chromosomes from different genomes. This species may, therefore, be seen to exemplify rather vividly the problem of the mechanism of diploidisation in a polyploid. What is known of the history and structure of chromosomes of the species contrasts so remarkably with the pattern of pairing observed at meiosis. Consequently it may be that the demonstration of the basis of the diploid-like behaviour in wheat will help to clarify related problems in other species.

3. MATERIAL

(i) *The haploids*

This work has principally involved the study of various kinds of haploids in *Triticum æstivum* variety Holdfast ($2n=42$). *T. æstivum* is a hexaploid so it would perhaps be strictly correct to use the term "polyhaploid". However, for simplicity, particularly in describing haploid plants with aneuploid numbers the basic term "haploid" seems preferable. It is applied to describe plants with essentially the gametic chromosome constitution.

Some of the haploids arose in material being used to add specific pairs of chromosomes of rye, *Secale cereale*, to the full complement of wheat chromosomes (Riley and Chapman, 1958*a*). Others occurred in the progenies of plants being used to

replace single pairs of wheat chromosomes by single pairs from rye (Riley and Bell, 1958). The derivations of all the haploids studied are shown in table 1.

All the haploids arose, by female parthenogenesis, from parents in which there had been either 20 or 21 bivalents as well as some univalents at meiosis. It is reasonable to suppose that the chromosomes in bivalents had segregated regularly and the univalents irregularly. Consequently, it can be assumed that a haploid,

TABLE 1

The origins of the haploids

Plant	Chrom. no.	Parent genotype	Phenotype or chromosome complement
Euhaploids			
1	21	21″w+ 3′r	euhaploid
■	21	21″w+ 1′r	euhaploid
3	21	21″w+ 1′ r	euhaploid
4	21	21″w+ 1′ r	euhaploid
5	21	21″w+ 1′ r telocentric	euhaploid
6	21	segregating population*	euhaploid
7	21	20″w+ 1′w+ 1′r telo.	no telo. present
8	21	21″w+ 2′r, long+ short telo. II	no telo. present
9	21	21″w+ 3′r	euhaploid
10	21	segregating population*	euhaploid
11	21	segregating population*	euhaploid
12	21	21″w+ 1′r	euhaploid
Nullihaploids			
nulli-I 1	20	20″w+ 1′wI+1′rIV	close to I mono.
nulli-I 2	20	20″w+ 1′wI+1′rIV	close to I mono.
nulli-XVII	20	20″w+ 1′wXVII+1′rIV	close to XVII mono.
nulli-V 1	▫▫	20″w+ 1′wV+1′rIII	All very short, well tillered, with very small, compact spikes, matching nulli-V
nulli-V 2	20	20″w+ 1′wV+ 1′rII	
nulli-V 3	20	20″w+ 1′wV+1′rIII	
nulli-V 4	20	20″w+ 1′wV+1′rIII	
nulli-V 5	20	20″w+ 1′wV♀ pollinated by *Aeg. caudata*	
Other aneuhaploids			
iso-V haploid	21	20″w+ 1′wV ♀ pollinated by 21″w+ 1″rV	
nulli-I rye-IV	21	20″w+ 1′wI+1′rIV	A clear effect of rye IV on spikelet morphology
Addition haploids			
rye-II haploid	▬	21″w+ 1′rII	Like rye II addn.
rye-III haploid	22	21″w+ 1′rIII	Like rye III addn.

′=univalent w=wheat

″=bivalent r=rye

* In the populations derived, after some generations without selection, from plants with 21″ wheat+7′ rye.

which had the same number of chromosomes as there had been bivalents in its parent, had received chromosomes from bivalents only. Thus, 21-chromosome haploids, from parents with all the wheat pairs plus a number of unpaired rye chromosomes, must have had the full gametic constitution of wheat and no rye chromosomes. Plants with this constitution, none of which showed any of the phenotypic effects expected from the presence of rye chromosomes, are here called "euhaploids".

A number of 20-chromosome plants occurred in the progenies of parents with 20 wheat bivalents plus a wheat and a rye univalent. The parents had been derived from crosses between 41-chromosome plants, monosomic for a specific wheat chromosome,

and 44-chromosome plants with the disomic addition of a specific rye chromosome to the full wheat complement. Consequently it was known which wheat and which rye chromosomes were unpaired. Further, the wheat chromosome deficient from 20-chromosome " nulli-haploids " corresponded to the one which was monosomic in the original parent. Thus the chromosome composition of the nulli-haploid could be accurately specified.

The wheat monosomics from which the nulli-haploids were ultimately derived were extracted during work with the variety Holdfast. However, chromosome numbering in wheat is based on the designations given to the monosomic lines in the variety Chinese Spring (Sears, 1954). Two of the Holdfast monosomics, I and V, to which nulli-haploids are related, have been checked and confirmed against the Chinese Spring numbering. The chromosome deficient from the other nulli-haploid has been shown by crossing possibly to be chromosome XVII but this has not been finally confirmed. Before its homology with V of Chinese Spring was established chromosome V of Holdfast was known as HH (Riley and Chapman, 1958*b*; Riley, Chapman and Kimber, 1960).

" Addition haploids " with 22 chromosomes were obtained from plants with 21 wheat bivalents plus a specific rye univalent. They were evidently formed by the parthenogenetic functioning of eggs which had received the rye univalent as well as the regularly segregating wheat chromosomes. Both addition haploids displayed phenotypic features characteristic of the particular rye chromosome present.

One 21-chromosome haploid was derived from a 42-chromosome parent which was monosomic both for chromosome I of wheat and for chromosome IV of rye. Since it possessed a phenotype characteristic of the presence of the rye chromosome IV, this chromosome must have been included, and chromosome I of wheat excluded. This plant may be conveniently referred to as a nulli-I rye-IV " substitution haploid."

Finally an " iso-V haploid " with 20 normal chromosomes, plus an isochromosome, was obtained when a wheat plant monosomic for chromosome V was pollinated with the pollen of a 44-chromosome rye chromosome disomic addition plant. The haploid apparently arose from an unfertilised egg which had received, in addition to the 20 normally segregating chromosomes, the isochromosome of the long arm resulting from the misdivision of the chromosome V univalent.

The euhaploids were collected and examined over a period of five years and the nulli-V haploids over a period of three years.

(ii) *Other material*

In testing the effects of each individual wheat chromosome on meiotic pairing use has been made of the series of 21 monosomic lines developed in *T. æstivum* variety Chinese Spring by Dr E. R. Sears. Other species used in this work were:

Secale cereale L. variety King II ($2n=14$).
Ægilops longissima Schweinf. and Muschl. ($2n=14$).
Ægilops speltoides Tausch *ligustica* Savign. ($2n=14$).
Ægilops cylindrica Host. var. *typica* Eig. ($2n.=28$).

4. CHROMOSOME PAIRING IN HAPLOIDS AND HYBRIDS

All cytological observations were made on Feulgen-orcein preparations. Squashes of pollen mother cells from anthers fixed in acetic alcohol were used in meiotic studies.

Chromosome pairing at first metaphase of meiosis has been studied in all the haploids examined and data from the cells scored have been presented in two ways. The mean pairing per cell (tables 2 and 4)

shows the general pattern of behaviour of each plant. In addition the same records have been presented as the frequencies of total chromosome associations per cell (tables 3 and 5). The totals of associations in each cell were obtained by summing the number of bivalents, trivalents and quadrivalents. They, therefore, indicate the number of groups of chromosomes within which pairing occurred and the frequency with which various levels of pairing were achieved.

The chromosome pairing in euhaploids provides a standard against which to compare the behaviour of other types of haploid plant. In

TABLE 2

*Mean pairing at first metaphase of meiosis in haploids**

Plant	Chrom. no.	Cells	Bivalents	Proportion closed bivalents	Trivalents
euhaploid 1 . .	21	100	1·39±0·09	—	0·07
euhaploid 2 . .	21	100	1·61±0·06	0·02	0·01
euhaploid 3 . .	21	100	1·69±0·12	0·02	0·05
euhaploid 4 . .	21	50	0·60±0·12	—	—
euhaploid 5 . .	21	50	0·72±0·10	—	—
euhaploid 6 . .	21	50	0·90±0·14	—	—
euhaploid 7 . .	21	50	1·02±0·09	0·02	0·02
euhaploid 8 . .	21	50	0·80±0·11	—	—
euhaploid 9 . .	21	50	0·78±0·09	0·03	0·02
euhaploid 10 . .	21	50	0·72±0·11	0·03	0·04
euhaploid 11 . .	21	50	0·82±0·11	—	—
euhaploid 12 . .	21	50	1·56±0·16	—	0·04
nulli-I haploid 1 .	20	100	0·66±0·07	—	—
nulli-I haploid 2 .	20	100	0·96±0·08	0·01	0·02
nulli-XVII haploid .	20	60	1·70±0·06	0·04	—
iso-V haploid . .	21	100	0·90±0·07	—	—
nulli-I rye IV haploid	21	50	0·88±0·05	0·04	—
rye-II haploid . .	22	40	1·25±0·13	—	0·08
rye-III haploid .	22	100	1·01±0·07	—	0·01

* The residue of chromosomes, after those in bivalents and tivalents, were univalents.

the twelve euhaploids examined, the mean bivalent frequency per cell ranged from 0·60, in euhaploid 4, to 1·69 in euhaploid 3. Trivalents were recorded in six plants but the highest mean frequency was only 0·07 per cell. Chromosome pairing was thus very low and the differences between the euhaploids are readily attributable to environmental differences. Mean pairing of this order has been widely reported in 21-chromosome haploids of *T. æstivum* (Riley and Chapman, 1957), and none of the present plants was at all atypical.

There were never more than five associations of synapsed chromosomes in any euhaploid cell. Indeed the presence of more than three associations was rare and there was never more than one trivalent per cell.

Thus there was very little pairing in these euhaploids of *T. æstivum*, despite the genetical similarity of homœologous chromosomes and despite the structural similarity of chromosomes of the component genomes. The low level of homœologous pairing (Sears and Okamoto, 1957) in 21-chromosome euhaploids is even more remarkable than its complete absence from 42-chromosome euploids. In the latter the strong attraction between complete homologues diminishes the possibility of homœologous association, but there can be no differential affinity to

TABLE 3

The distribution of cells with various totals of bivalents plus trivalents in haploids

Plant	Cells	Percentage of cells with various numbers of associations							
		0	1	2	3	4	5	6	7
euhaploid 1 . .	100	15	45	28	10	2	0	0	0
euhaploid 2 . .	100	16	22	46	13	2	0	0	0
euhaploid 3 . .	100	17	29	30	16	8	0	0	0
euhaploid 4 . .	50	60	24	12	4	0	0	0	0
euhaploid 5 . .	50	40	48	12	0	0	0	0	0
euhaploid 6 . .	50	44	34	12	8	2	0	0	0
euhaploid 7 . .	50	18	62	20	0	0	0	0	0
euhaploid 8 . .	50	38	46	14	2	0	0	0	0
euhaploid 9 . .	50	38	50	8	4	0	0	0	0
euhaploid 10 . .	50	42	50	2	6	0	0	0	0
euhaploid 11 . .	50	36	50	10	4	0	0	0	0
euhaploid 12 . .	50	18	30	36	12	2	2	0	0
nulli-I haploid 1 .	100	46	45	6	3	0	0	0	0
nulli-I haploid 2 .	100	26	54	19	0	1	0	0	0
nulli-XVII haploid .	60	10	35	29	18	5	1	0	1
iso-V haploid . .	100	26	60	0	1	0	0	0	0
nulli-I rye-IV haploid	50	24	56	20	0	0	0	0	0
rye-II haploid . .	40	17	47	27	9	0	0	0	0
rye-III haploid .	100	19	62	18	1	0	0	0	0

reduce homœologous pairing in euhaploids. Homœologous affinity is thus only weakly expressed in hexaploid wheat.

The two nulli-I haploids and the nulli-XVII haploid fit very well into the range of pairing patterns found in the euhaploids. Consequently the deficiency of chromosomes I and XVII had no major influence on meiosis. It therefore appears that they carry no genes which exercise any significant control over haploid meiosis.

Similarly the nulli-I rye-IV haploid condition did not produce any marked modification of meiosis, compared with the euhaploid state.

Lastly amongst this group of aneuhaploids, the two 22-chromosome plants, with rye chromosomes II and III respectively, did not deviate

from the narrowly circumscribed behaviour of the euhaploids. The presence of the extra chromosome, in each case, had not affected pairing behaviour, and the rye chromosome was always a univalent.

The striking conclusion, which emerges from this examination of a number of haploids with abnormal genetic constitutions, is that meiosis in wheat seems to follow a course which is narrowly restricted. The overall genetic organisation of meiosis of hexaploid wheat is such that, in plants at the haploid condition, a particular meiotic pattern, with only a slight amount of pairing, occurs. Moreover, even though the haploid genotype is greatly modified by the addition, removal or replacement of particular chromosomes, meiosis still follows the same course. The organisation of meiosis in wheat is, therefore, remarkably stable.

TABLE 4

Mean chromosome pairing at first metaphase of meiosis in nulli-V haploids.

Plant	Cells	Univ.	Bivalents			Triv.	Quad.
			Rod	Ring	Total		
nulli-V haploid 1	75	8·99±0·53	3·17±0·25	1·00±0·12	4·17±0·28	0·86±0·27	0·02
nulli-V haploid 2	30	8·00±0·57	2·37±0·20	0·80±0·13	3·17±0·22	1·80±0·21	0·07*
nulli-V haploid 3	33	6·40±0·39	1·94±0·22	1·61±0·21	3·55±0·31	2·00±0·20	0·12
nulli-V haploid 4	44	6·46±0·34	2·32±0·20	1·68±0·20	4·00±0·22	1·73±0·16	0·09
nulli-V haploid 5	36	6·53±0·31	2·75±0·23	0·97±0·17	3·72±0·24	1·83±0·21	0·06*†

* Plus one association of five. † Plus one association of six.

There was a startling change, however, when chromosome V was deficient from the haploid complement (table 4). Nulli-V haploids had means of between 3·17 and 4·17 bivalents per cell. Thus they had more than twice as many bivalents as most euhaploids. Moreover much higher proportions of the bivalents in nulli-V haploids were closed rings.

In addition there were means of from 0·86 to 2·00 trivalents per cell in nulli-V haploids and generally there were more trivalents in plants of this constitution than there were bivalents in euhaploids. The trivalents were mostly chains, but tri-radials and " pan-handles " also occurred. There were also infrequent chain quadrivalents.

The maximum pairing observed was in a cell with five trivalents, two bivalents and one univalent. By contrast the highest observed in a euhaploid cell was five bivalents and 11 univalents. Whereas there were none, one, or two associations in most euhaploid cells, many cells had four, five, or six associations in the nulli-V haploids, and those with none, or only one or two, were very rare.

It is clear, from the contrasts between the nulli-V and all other haploid conditions, that chromosome V must carry a gene, or genes,

with a powerful influence on meiotic pairing. The effect of the presence of the V gene, in a normal wheat plant, is to diminish associations. This immediately raises the question of the relationships between the chromosomes which pair in its absence. It may be assumed, since the evidence to the contrary is limited (McClintock, 1933; Levan, 1942; Reiger, 1957), that meiotic chromosome pairing which accompanies chiasma-formation usually takes place between structurally and genetically corresponding segments. The increased pairing in nulli-V haploids must, therefore, imply some segmental homology between the associated chromosomes.

There are two explanations which might account for this segmental homology. First the paired regions might be randomly distributed duplicate segments between which synapsis is normally inhibited.

TABLE 5

The distribution of cells with various totals of bivalents, trivalents, quadrivalents and higher associations in nulli-V haploids.

Plant	Cells	Percentage of cells with from 0-8 associations								
		0	1	2	3	4	5	6	7	8
nulli-V haploid 1	75	0	0	4	1	20	44	26	4	1
nulli-V haploid 2	30	0	0	3	10	17	30	30	10	0
nulli-V haploid 3	33	0	0	0	0	15	17	55	10	3
nulli-V haploid 4	44	0	0	0	0	5	27	48	16	4
nulli-V haploid 5	36	0	0	0	0	6	31	55	8	0

Alternatively, the paired regions might be in genetically and cytologically corresponding, homœologous, chromosomes from the different genomes.

One of the most prominent considerations which lends favour to the latter hypothesis is the high frequency of trivalents. There were as many as five in some nulli-V haploid cells. The formation of trivalents is a likely outcome of the association of all three homœologous chromosomes but it is improbable that so much structural triplication should be present, unrelated to polyploidy. Also there were very rarely more than seven associations per cell and this can be satisfactorily explained if pairing occurs almost entirely within the seven homœologous groups and rarely between groups. However a small amount of homology must be postulated between groups to account for the rare cells with a quadrivalent or with eight associations. It is extremely improbable that structural duplications, unrelated to the polyploid nature of the plant, would be so distributed as to cause meiotic pairing which coincides so admirably with that expected from homœologous association. Consequently it seems reasonable to conclude that an important influence of chromosome V in euhaploids is greatly to restrict pairing between homœologues. It is natural therefore, to

enquire whether it exercises a similar function in 42-chromosome hexaploid wheat.

Meiosis was examined in 40-chromosome nullisomics of Holdfast wheat which were deficient for both members of the V pair. These were found to have a multivalent in most cells, and sometimes several per cell (fig. 1). Quadrivalents were slightly more common than

(*a*) (*b*)

FIG. 1.—Camera lucida drawings of first metaphase of meiosis in pollen mother cells of *T. æstivum* (*a*) nullisomic for chromosome V showing 15 bivalents, one ring of four and one chain of six, (*b*) normal hexaploid showing 21 bivalents. (Present magnification ×1150.)

trivalents and there were also associations of five and six chromosomes (table 6). In many cells there were also univalents which were not the residue of odd-number multivalents. The meiotic behaviour of these nullisomics was clearly consistent with the notion that homœologous pairing was occurring.

TABLE 6

Mean pairing at first metaphase of meiosis in euploid and nulli-V T. æstivum.

Plant	Cells	Univ.	Biv.	Triv.	Quad.	5—	6—
euploid	200	0·14	20·93	—	—	—	—
nulli-V	60	1·41	17·22	0·47	0·59	0·02	0·05

Thus the absence of chromosome V changed wheat from a regular and stable bivalent-forming plant, typical of the classical concept of an allopolyploid species, into one behaving more like a segmental allopolyploid or indeed like an autopolyploid. Therefore a genetic mechanism on chromosome V normally limits pairing in this way, and furthermore, there is no independent system on any other chromosome which can make up for the absence of the V-gene. However, it might be that the critical gene, or genes, on V form part of a complementary system, involving genes in other linkage groups. Removal of any other chromosome involved in the system would then cause a breakdown

in the normal organisation of meiosis, similar to that which results from the deficiency of V.

In testing this, use was made of the knowledge that the increased pairing in V-deficient material shows well in hybrids between wheat and rye, *Secale cereale*, lacking V (Riley, Chapman and Kimber, 1959). Hybrids with 27-chromosomes, deficient for any wheat chromosome, can be produced by crossing the appropriate wheat monosomic, as female, with rye. The univalent wheat chromosome is excluded from about three quarters of the embryo sacs of the monosomic parent, so

TABLE 7

Mean chromosome pairing at first metaphase of meiosis in T. æstivum × S. cereale *hybrids deficient in turn for each wheat chromosome* *

Chromosome number	Chromosome deficient	Cells	Bivalents	Trivalents	Quadrivalents
28	—	300	0·48	—	—
27	I	100	0·11	—	—
27	II	100	0·15	—	—
27	III	100	0·14	—	—
27	IV	100	0·37	0·01	—
27	V	200	3·36	0·88	0·08
27	VI	100	0·31	—	—
27	VII	100	0·40	—	—
27	VIII	100	0·32	0·01	—
27	IX	100	0·75	—	—
27	X	100	0·33	0·01	—
27	XI	100	0·06	—	—
27	XII	100	0·49	—	—
27	XIII	100	0·12	—	—
27	XIV	100	0·22	—	—
27	XV	100	0·71	—	—
27	XVI	100	1·00	—	—
27	XVII	100	0·77	—	—
27	XVIII	100	0·86	—	—
27	XIX	100	0·47	—	—
27	XX	100	0·23	—	—
27	XXI	100	0·29	—	—

* The residue of chromosomes, not in bivalents, trivalents or quadrivalents, were univalents.

that both 27- and 28-chromosome hybrids can be obtained from the same parents.

Hybrids were produced between *T. æstivum*, Chinese Spring and *Secale cereale*, deficient in turn for each of the 21 chromosomes of wheat. Examination of first metaphase of meiosis showed that only in the hybrids deficient for chromosome V was there a serious difference between the pairing of 27- and 28-chromosome plants (table 7). Thus the removal of no other wheat chromosome results in a modification of pairing comparable with that arising from the absence of V. This chromosome is, therefore, unique in having the property of preventing homœologous association.

Simultaneous evidence of the responsibility of chromosome V for the diploid-like behaviour was provided independently of the present

work by Sears and Okamoto (1958). In investigating hybrids between *T. æstivum* monosomics and diploid wheat, they found that there was much higher pairing in those hybrids from which chromosome V was deficient. The increased pairing could only be interpreted as arising from homœologous conjugation. Thus the evidence from V nullisomics, from nulli-V haploids and from V-deficient hybrids fits together well.

Further information on the localisation of pairing control was obtained from the iso-V haploid plant (table 2). This had 20 normal chromosomes plus the isochromosome for the long arm of V, and was completely deficient for the short arm. Chromosome V is markedly heterobrachial (Sears, 1954) having one arm about twice the length of the other. Meiosis in the iso-V haploid was similar to that in euhaploids, removal of the short arm producing no alteration in pairing. Moreover, 41-chromosome plants, with V represented by a monosomic telocentric for the long arm, regularly form 20 bivalents and a univalent at meiosis, and never make multivalents. In addition 42-chromosome plants disomic for the long telocentrics form only bivalents. Consequently the effective region of the chromosome is not restricted to the short arm. Thus it may be that a part of the long arm alone is responsible, or that pairing limitation results from the joint activity of genes in both arms.

It has not so far been possible to obtain a plant with the telocentric for the short arm of V but deficient for the long arm. However, the short telocentric can be maintained in 42-chromosome plants, which also have one complete chromosome. No segregants deficient for the complete chromosome are produced, presumably because of the poor competitive ability of pollen grains carrying the short telocentric. Nevertheless plants with the complete chromosome V plus the short telocentric can be pollinated by another species and use made of the embryo sacs with the telocentric to produce hybrid individuals deficient for the long arm. Also normal monosomics, and plants disomic for telocentrics of the long arm can be used to make other hybrids for comparison. Hybrids with the complete chromosome V, deficient for V, and with either the long or the short arm telocentric, can thus be compared.

The most satisfactory comparisons would be based on hybrids involving a diploid such as rye or *A. longissima*, as the male parent, but results are so far available only from crosses based on *Ægilops cylindrica*, an allotetraploid with the genomic constitution CCDD. Meiotic pairing has been compared in the four types of hybrid (table 8), and there were almost seven bivalents per cell in normal 35-chromosome hybrids, carrying the complete chromosome V. This was due to the two D genomes derived by each parent from *A. squarrosa* (McFadden and Sears, 1946). However, the only multivalents were the rather rare trivalents representative of the intergenome pairing found in euhaploids of wheat.

Hybrids deficient for V had a considerably greater frequency of multivalents with no reduction in the bivalent frequency. Thus the effects of chromosome V, on meiotic pairing, were detectable in these hybrids, involving a polyploid, just as they were in those with rye (table 7) and with *A. longissima* (table 9), both diploid species. Nevertheless the increase in pairing was less dramatic in this example perhaps because of the disomic condition of the D genome chromosomes or because of the possible effect of genetic diploidising influences in the *A. cylindrica* genotype.

There was a clear difference between the hybrids carrying the long and the short arm telocentrics respectively. Those deficient for the long arm much more nearly approached the behaviour of the

TABLE 8

The mean pairing at first metaphase of meiosis in hybrids of T. æstivum × A. cylindrica *with the complete chromosome complement of* T. æstivum, *or with* T. æstivum *deficient for chromosome V or either its long or its short arm*

Status of *T. aestivum* complement	Chrom. No.	Cells	Univ.	Biv.	Triv.	Quad.	5–
complete . .	35	40	20·89	6·80	0·17	—	—
deficient short V .	35	50	19·62	7·18	0·34	—	—
deficient long V .	35	50	16·40	6·44	1·26	0·46	0·02
deficient V . .	34	100	13·16	7·41	1·36	0·36	0·10

V-deficient hybrids, especially in the high frequency of multivalents. By contrast those deficient for the short arm were little different from the euploid hybrids with the complete chromosome, having no multivalents higher than trivalents.

From this it seems that the diploid-like meiotic behaviour of wheat is principally controlled by one or more genes on the long arm of chromosome V. As a note of caution, however, it should be indicated that the long arm-deficient hybrids with *A. cylindrica* did not fully reach the level of pairing found in hybrids lacking the complete chromosome. However, the difference was small, a mean of 2·24 fewer chromosomes paired, and may have no genetic significance.

5. ORIGIN OF THE DIPLOIDISING MECHANISM

There is no direct evidence concerning the origin of the pairing control exercised by chromosome V, so that discussion must be mainly speculative. First of all, however, an indisputable preliminary point is that chromosome V is in the B genome of hexaploid wheat (Sears, 1958) and consequently it has a homologue in tetraploid wheat. Hybrids between tetraploid and hexaploid wheats do not produce, amongst their derivatives, any segregants with intergenome associations. Consequently there must be alleles with similar effects at both levels of polyploidy.

The diploidising effect of chromosome V may result from an activity which the chromosome also performed in its original diploid background. Alternatively gene mutation subsequent to the incorporation of the chromosome in polyploid wheat may have altered its activity. If chromosome V caused diploid-like behaviour without modification, then amongst the probable contributors of the B genome a species should be found producing the same effect as V.

The B genome probably came to polyploid wheat from a species of the Sitopsis section of *Ægilops* (Sarkar and Stebbins, 1956). This

TABLE 9

The mean chromosome pairing at first metaphase of meiosis in the hybrids T. æstivum × A. speltoides *and* T. æstivum × A. longissima, *with and without chromosome V of* T. æstivum.

Plant	Chrom. number	Chrom. V	Cells	Univ.	Biv.	Triv.	Quad.	5—
× *A. speltoides*	28	present	50	6·04	6·64	1·88	0·76	—
× *A. speltoides*	28	present	50	3·40	6·14	2·20	1·30	0·04
× *A. speltoides*	27	absent	30	6·13	5·23	1·76	1·23	0·03
× *A. speltoides*	27	absent	30	6·90	6·10	1·33	0·93	0·03
× *A. longissima*	28	present	100	23·90	1·96	—	—	—
× *A. longissima*	27	absent	100	7·50	7·58	0·70	0·56	—
× *A. longissima*	27	absent	100	9·80	6·45	0·87	0·41	—

includes four species, all diploids, namely *A. speltoides*, *A. bicornis*, *A. longissima* and *A. sharonensis*, although the last two should perhaps be lumped (Tanaka, 1955). Riley, Unrau and Chapman (1958) have already pointed out that *A. speltoides* behaves quite differently from the other Sitopsis species in hybrids with tetraploid wheat. Hybrids with *A. speltoides* have high pairing at meiosis and show a breakdown in the isolation of homœologous chromosomes. This does not happen in hybrids with any other Sitopsis species in which, indeed, there is very little chromosome pairing at all. Superficially, therefore, the species other than *A. speltoides* seem to produce effects on chromosome pairing analogous to those caused by wheat chromosome V.

An attempt was therefore made to understand the relationships between the effect on homœologous pairing of chromosome V and of the genotypes of two Sitopsis species (Riley, Kimber and Chapman, 1961). For this purpose hybrids, with and without chromosome V, were made between *T. æstivum* and either *A. speltoides* or *A. longissima*, in the way previously described for chromosome-deficient wheat-rye hybrids.

A. speltoides and *A. longissima* are very similar in chromosome structure, and hybrids between them have good meiotic pairing although they differ by a reciprocal translocation. However, their

hybrids with *T. æstivum* behaved quite differently (table 9). In 28-chromosome hybrids with *A. speltoides* there was very high pairing, with many trivalents and quadrivalents. The normal barrier between homœologues was broken down. There was little difference between these and the 27-chromosome *A. speltoides* hybrids, deficient for chromosome V. Thus it appears that the *A. speltoides* genotype inhibits the influence of chromosome V, so that the absence of V produces no effect in the presence of the *A. speltoides* genotype.

There was little chromosome pairing in 28-chromosome hybrids with *A. longissima*, consequently this genotype does not suppress the V effect. By contrast 27-chromosome, V-deficient, hybrids with *A. longissima* showed considerable homœologous pairing, absence of V from the hybrid acting in the same way as its absence from a haploid. Significantly, therefore, the *A. longissima* genotype cannot compensate for the absence of V in preventing homœologous association and so it clearly does not function in the same way as chromosome V. *A. bicornis* similarly cannot compensate for the deficiency of V (Riley, unpublished). Thus, assuming *A. sharonensis* to equal *A. longissima*, all the Sitopsis species which do not suppress the V effect are also not equivalent to V in activity. It is unlikely, therefore, that the V condition came, without modification, from the donor of the B genome in which it might conceivably have been responsible for restricting pairing between small structural duplications. On the contrary the effect is probably produced by one or more loci on the critical chromosome, altered or unmasked by mutation subsequent to the formation of the first polyploid.

The nature of the mutation involved is open to a number of possible explanations, but it is most readily conceived as occurring at a single locus, although a more complex origin cannot be excluded. The locus on V, responsible for the restriction of intergenome pairing, may also be concerned with the differences in the meiotic behaviour, in hybrids, of *A. speltoides* and the other Sitopsis species. On this hypothesis the three different pairing effects would be due to alleles at the same locus.

Alternatively a mutant on V may have arisen at a locus at which *A. speltoides* and the remaining species are not different. The cause of their different behaviour in hybrids would then reside elsewhere in their genotypes. This second hypothesis has certain disadvantages if, as is likely, *A. speltoides* is the B genome donor. For then mutations elsewhere must be postulated to have overcome the inhibitory influence which the *A. speltoides* genotype has over the normal functioning of the chromosome V mechanism.

Thirdly *A. longissima* and *A. speltoides* may differ at the V locus, *A. speltoides* carrying a gene there similar in action to that on V of wheat. However, the effect of this may be inhibited in *A. speltoides* by genes elsewhere in the genotype, mutation of which in the polyploid removed the inhibition, so that the V condition took over the regulation of the diploid-like behaviour.

If, however, the regulatory effect of V arose, as postulated, by mutation either on V or elsewhere, then the significant gene, or genes, must subsequently have been fixed by selection. This probably occurred in the tetraploid wheats, all of which are diploidised. The already adjusted genotype was then passed on to the hexaploids. Selection in the tetraploid would readily favour genetic variants which precluded the production of homœologous multivalents, the cause of lower fertility and irregular genetic segregation.

6. FUNCTIONING OF THE CHROMOSOME V GENE

There is little difference between the frequency and distribution of the chiasmata linking paired chromosomes in euploid and nullisomic V plants. Thus the effect of chromosome V is probably not directly related to chiasma formation. In addition, although the analysis of early prophase of meiosis is unsatisfactory in wheat, the chromosomes of euploid plants seem to be fully paired throughout their length. Consequently, there are probably few regions with unsatisfied pairing where genetical alteration in the size of the pairing blocks, or the intimacy of association, could give rise to the increased conjugation observed in the absence of V.

It may be, therefore, that the influence of chromosome V is on the forces which attract chromosomes together and which initiate pairing. In euploid wheat the attraction between complete homologues, which are more or less identical structurally, is sufficient to cause very regular bivalent formation. However, there is less attraction between homœologues, which are somewhat divergent in structure, and the attraction is normally insufficient to cause them to pair. Chromosome V thus appears to control pairing by reducing attraction to a level below which homœologous pairing cannot be completed. It seems likely, in view of the increased effect in its absence, that the chromosome V gene inhibits positively acting processes, responsible for pairing, which are determined elsewhere in the genotype.

The effect of chromosome V could thus be described as the genetic enhancement of differential affinity (Darlington, 1937). Its development was probably dependent upon the occurrence, in the raw polyploid, of greater attraction between homologues than between homœologues. This is presumably a system, therefore, which could not evolve in a true autopolyploid where each chromosome is equally attracted to a number of identical homologues.

7. THE DIPLOIDISATION OF POLYPLOIDS

There are many polyploid species of undoubted hybrid origin which only form bivalents at meiosis. These are the allopolyploid forms of classical theory, which ideally arise from hybrids between two or more species, in which the chromosomes of the parents are so different that

they are unable to pair, or form only a small number of loosely associated bivalents. Chromosome doubling in such hybrids supposedly gives rise to polyploids in which each chromosome pairs only with its identical partner. Meiosis is therefore regular and there is disomic inheritance although often with many duplicate genes.

To determine the likelihood of the fullest expression of allopolyploid behaviour occurring directly, simply as a consequence of chromosome doubling in hybrids, well established examples of allopolyploidy can be considered. Two comparisons are possible, the first being made between the chromosome behaviour of the natural polyploid species and that of the synthetic polyploid derived from hybrids between its putative diploid parents. Secondly, chromosome pairing can be compared in haploids of tetraploid species and in the initial hybrid between the putative diploid parents. That is, the established polyploid can be compared with the " raw " polyploid, or the true haploid with the synthetic haploid.

The hybrid between *Nicotiana sylvestris* and *Nicotiana tomentosiformis*, close relatives of the diploid parents of *Nicotiana tabacum*, a tetraploid, has a mode of three and a maximum of seven bivalents (Goodspeed, 1934). By contrast the haploid *N. tabacum* normally has a mode of zero and a maximum of three bivalents (Lammerts, 1934). This suggests that the diploidisation of tobacco was apparently not automatically achieved on chromosome doubling.

Similar methods may be applied to the tetraploid species *Poa annua* which probably combines the genomes of the two diploids *P. infirma* and *P. supina* (Tutin, 1952, 1957). *P. annua* forms only bivalents at meiosis (Hovin, 1958) but in the synthetic tetraploid derived from the hybrid between the putative parents there were multivalents (Tutin, 1957). Thus *P. annua* has evolved towards the diploid-like condition, rather than achieving it directly with polyploidy.

Again, *Brassica juncea*, a tetraploid, shows evidence of diploidisation subsequent to the origin of the polyploid since there is more pairing in hybrids between its putative parents, *B. campestris* and *B. maja*, than in haploid *B. juncea* (Ramanujam and Srinivasachar, 1943).

A further example is provided by the tetraploid New World cottons, in one species of which, *Gossypium barbadense*, the haploids rarely form bivalents. However the F1 hybrid between species related to the original diploid parents, *G. arboreum* and *G. thurberi*, averages eight bivalents per cell and the raw tetraploid has some multivalents (Skovsted, 1937; Beasley, 1942).

Finally, taking a species closely related to wheat, the haploid of *Ægilops ovata*, a tetraploid, has considerably less pairing than the hybrid between its parents (Matsumura, 1940; Kihara, 1937). It is therefore clear that in other polyploid species, in addition to wheat, completely regular meiosis, with pairing restricted to identical partners, and disomic inheritance, has developed after the origin of the polyploid condition.

Indeed it is probably often not realised that some of the synthetic forms, which have been regarded as good examples of allopolyploid behaviour, are not completely stable at meiosis and make multivalents. Into this class must go *Raphano-brassica* (Richharia, 1937) and *Primula kewensis* (Upcott, 1939).

Further, the use of synthetic polyploids to illustrate the immediately stable behaviour of allopolyploids must be invalid when one of the parents is already polyploid (Stebbins, 1950). The reason for this is that a genetic condition leading to the elimination of multivalents, and effective in the new synthetic, may already be present in the polyploid parent. This difficulty is illustrated by the *T. æstivum* × *Secale cereale* (Triticale) and *T. æstivum* × *A. longissima* allopolyploids, in which multivalents are never formed in the presence of wheat V, but do occur in its absence (Riley, unpublished).

It must not be supposed, however, that the direct origin of a purely bivalent-forming polyploid, from diploids, can never occur. For example the haploid of *Brassica napus* has about the same meiotic pairing as the F1 hybrid between its probable parents *B. campestris* and *B. oleracea* (Olsson and Hagberg, 1955; U, 1935). Also the raw tetraploid obtained from the hybrid is meiotically regular, like the natural *B. napus* (Frandsen, 1947). In addition, the synthetic *Galeopsis tetrahit* produced by Müntzing (1930) from *G. pubescens* and *G. speciosa* appears to have been immediately regular and stable at meiosis. Nevertheless, although such stable forms can occasionally occur they seem to be less common. Most forms which are sufficiently similar to form viable hybrids also have chromosomes capable of conjugation in their allopolyploid derivatives.

Since the diploidisation of polyploids appears to have been common in the evolution of higher plants the processes involved may be enquired into. Two hypotheses have been proposed but, until the wheat example, little direct evidence was available. One suggested possibility was that the basic diploid chromosome sets, combined in the polyploid, diverged from one another in structure (Darlington, 1937; Stebbins, 1950). Structural alterations which impeded meiotic conjugation between homœologous chromosomes, from different genomes, were selected and became homozygous because of their influence on fertility through reduced multivalent formation. The accumulation of many such structural alterations, it was considered, would eventually lead to the complete differentiation of the chromosomes of the different genomes and so to a purely bivalent-forming meiotic pattern. Such processes would inevitably be very slow and the young polyploid species, for much of its early and uncertain existence would be hampered by considerable infertility.

Although it is hard to prove, this process may have had some significance in polyploid evolution. However, its limited importance is perhaps illustrated by the frequency with which, by meiotic pairing in hybrids, the chromosomes of diploids can be matched with related sets

in polyploids. In this way the restricted extent of changes in structure, from the original diploid condition, can be revealed in polyploids which have clearly undergone diploidisation.

The diploidisation of polyploids may also be achieved by the selection of genetic variants with modified chromosome pairing. The situation in *T. æstivum* is an example of this, with selection for fertility and genetic stability, in the original tetraploid, probably leading to the fixation of the mutant condition. Such a process, with a simply inherited control over the patterns of conjugation, would be rapid and precise and the newly arisen polyploid would be exposed to the minimum period of reproductive inefficiency.

The basis of meiotic behaviour is not established in any other allopolyploid as it is in wheat. However, in the light of the results with wheat, work by Lammerts (1934) on haploids of *Nicotiana tabacum*, a diploidised tetraploid, may perhaps be tentatively re-interpreted. Lammerts found that a haploid from *coral*, a mutant form heterozygous for a translocation, had much higher pairing than haploids from normal parents. Apparently the *coral* haploid had arisen from a gamete with a duplication-deficiency segregation of the chromosomes involved in the translocation. Nevertheless, the extra pairing was much more than could be ascribed solely to the presence of the duplicated segment. Lammerts interpreted this as non-homologous pairing in a physiologically abnormal individual. It seems quite possible, alternatively, that the additional pairing involved homœologous chromosomes between which conjugation was normally inhibited by the activity of genes on the segment rendered deficient by the segregation of the *coral* translocation. This suggests, therefore, that there may be, in *N. tabacum*, a genetic regulation of the diploid-like behaviour similar to that in wheat.

A further example of genetic diploidisation, suggested to me by Mr G. Kimber, can perhaps be demonstrated in the tetraploid New World cottons. In the triploid hybrids between such a tetraploid, for example *Gossypium barbadense*, and either of its diploid ancestors, there is good pairing at meiosis between chromosomes of the genomes common to the diploid and the tetraploid. Consequently no major accumulation of structural modifications can have occurred in the tetraploid. Moreover, there is reasonably good pairing in hybrids between the two diploid ancestors, but in haploids of the tetraploid there is virtually no pairing (Beasley, 1942). Since the lack of pairing between homœologues in the haploid cannot be entirely due to structural differentiation it presumably has a genetic basis.

Thus it can be seen that in those polyploid species, inevitably crop plants, which have been most widely investigated, evidence of genetical diploidisation can be discerned. Indeed it may well be that systems like that in wheat will prove to be the usual method of achieving a diploid-like pattern of behaviour. However the combination of genetical and chromosome-structural diploidisation may also occur,

and some purely bivalent-forming types certainly arise directly without subsequent modification.

The preceding discussion has been concerned with polyploids which regularly form bivalents at meiosis and have disomic segregation ratios, that is polyploids in which each chromosome pairs with its single identical partner. It is important to distinguish these species from others which, while commonly forming only bivalents at meiosis, have polysomic segregation. In such species four or more partner chromosomes conjugate at random in pairs.

Such a case has been well documented in the hexaploid *Phleum pratense* (Nordenskiöld, 1945, 1953), European forms of which make 21 bivalents but show hexasomic inheritance. A similar example is provided by *Lotus corniculatus*, a tetraploid, in which cyanogenetic activity is inherited tetrasomically but which rarely forms multivalents at meiosis (Dawson, 1941).

This type of organisation, whilst affording regular chromosome segregation and high fertility, has less significance in evolution than that of polyploids with disomic segregation. In the latter, alleles at some of the duplicated loci can mutate away from their original function without detriment, since the function is continued at homœologous loci. The outline of this process has been illustrated in polyploid wheat by comparing the pattern of induced mutations in diploid, tetraploid and hexaploid species (MacKey, 1958). Thus evolutionary exploration can take place in a polyploid with disomic inheritance in a way not possible in a bivalent-forming autopolyploid, since in the latter selection of a mutant allele must lead to the eventual elimination of the pre-existing allele and to loss of the original function.

8. THE DIPLOIDISATION OF WHEAT AND WHEAT BREEDING

The introduction of genetic variation from many primitive species related to wheat is prevented because, in hybrids, wheat chromosomes do not pair with the chromosomes of the alternative parent. Riley and Chapman (1958*b*) reasoned that this lack of pairing may often be due to the same activity of chromosome V which prevents pairing between the wheat genomes. Intergeneric hybrids deficient for V were therefore investigated (Riley, Chapman and Kimber, 1959). Under these conditions increased pairing occurred, some of which must have associated wheat chromosomes and the chromosomes of the alternative parents, *S. cereale* and *A. longissima*. This was illustrated by the formation of quadrivalents, combining three wheat homœologues and the matching foreign chromosome, in hybrids deficient for V, although there was no allosyndetic pairing when V was present. Consequently removing chromosome V disposes of the initial barrier to the incorporation of much foreign variation in wheat.

Secondly, 40-chromosome, V-nullisomic plants which undergo intergenome pairing offer a means of repatterning the chromosome

structure of wheat. Recombination between homœologues leads to the formation of translocations relative to the original structure. The new states can be stabilised when made homozygous and chromosome V is restored, and the lines so produced may possess agricultural advantages either directly or as a consequence of the altered segregations following their use in hybridisation. Finally, intergenome pairing may make it possible to obtain beneficial genes with dose effects duplicated or triplicated on homœologous chromosomes, although they could normally be no more than disomic.

Knowledge of the diploidisation of *T. æstivum* can clearly extend the horizons of wheat breeding in direct and practical ways. However, further investigation is needed before appropriate methods can be devised for handling the material involved.

9. SUMMARY

1. The absence of one chromosome, number V, greatly modifies the meiotic pairing of haploids and nullisomics of wheat, *Triticum æstivum* ($2n=6x=42$). It is concluded that this chromosome carries a genetic structure responsible for the diploid-like meiotic and genetical behaviour of the hexaploid. In the presence of chromosome V the genetically and structurally equivalent chromosomes of the different genomes of wheat do not pair, but this isolation breaks down in its absence.

2. Evidence from a series of hybrids with *Secale cereale* shows that no similar modification of pairing occurs in the absence of any of the other 20 chromosomes. In addition results from hybrids between wheat and *Ægilops cylindrica*, deficient either for the complete chromosome V or for its long or short arm separately, indicate that the control is exercised by the long arm alone. Thus the genetic system is very localised and it may be that a single locus is responsible.

3. None of the diploid species from which chromosome V may have been derived produces a genetic effect on pairing like that of V. This suggests that the diploidising function of V arose by mutation after the origin of polyploidy in wheat, probably at the tetraploid level. It could have resulted either from mutation on V itself or from an unmasking mutation elsewhere. The mutant would probably have been favoured by selection for the improved fertility and genetic stability to which it led.

4. It is suggested the meiotic regulation operates through the modification of pairing attractions during prophase.

5. Many polyploids have acquired a purely bivalent-forming meiotic regime, with disomic inheritance, subsequent to the development of polyploidy. It seems likely that this may often have been the outcome of genetical, rather than chromosome-structural, modifications.

6. Manipulation of the genetic control of pairing, exercised by

chromosome V, may have important consequences for wheat improvement, both in the incorporation of variation from other species, and in the reorganisation of the genetic structure of wheat itself.

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NUCLEOLAR CONTROLLED SEGREGATION OF THE SEX CHROMOSOMES IN BEETLES

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1. INTRODUCTION

THE commonest method of diploid sex determination depends on the segregation of distinctive chromosomes which are homozygous in one sex and heterozygous in the other. Specific pairing followed by chiasma formation is both a sufficient and common means of achieving segregation in such systems. But chiasmata are not indispensable and they may be supplemented or replaced by other devices which allow the sex chromosomes to undergo regular segregation in the heterogametic sex. It is interesting to note, however, that in very few of the cases where chiasmata are lacking is bivalent formation also dispensed with.

Chiasmate and non-chiasmate associations between sex chromosomes are not always easy to distinguish. This applies to the unique parachute-type sex bivalent found in the males of at least twenty families of the *Coleoptera* (Smith, 1953). These bivalents consist of a relatively large metacentric X chromosome associated with an invariably smaller and often minute y_p chromosome. Smith (1951) has tentatively advanced the view that these unequal chromosomes are associated at metaphase by two terminal chiasmata. One obvious factor which suggests that this may not be the case is the very small size of the y chromosome.

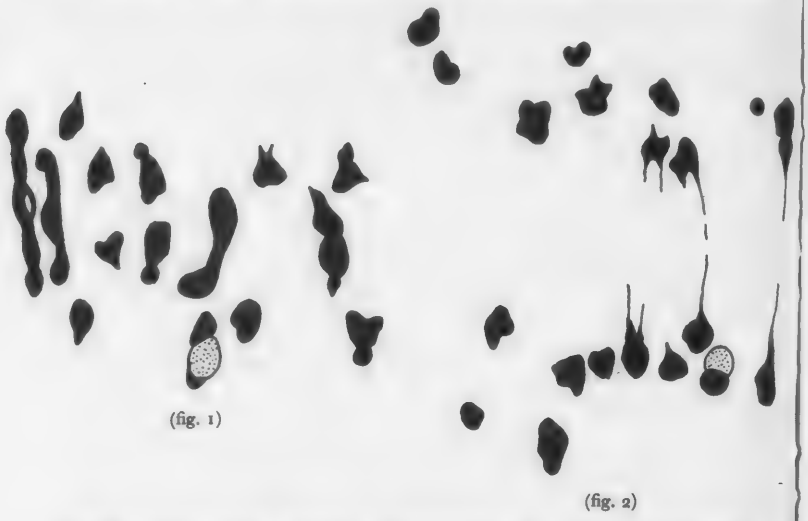
In 1957 we reported a multiple sex-chromosome system in the cellar beetle, *Blaps mucronata* (*Coleoptera: Tenebrionidæ*), where segregation depended, in part, on an association between the sex chromosomes and a persistent nucleolus (Lewis and John, 1957). This case has led us to consider whether a similar mechanism operates also in Xy_p-coleopterans which belong to the presumed ancestral type.

To test this we have examined males of the two-spotted ladybird (*Adalia bipunctata*) and the seven-spotted ladybird (*Coccinella septempunctata*), in which the Xy_p metaphase bivalent is among the largest and the clearest we have seen. Males of *Tenebrio molitor* have also been used because, while the metaphase bivalent is less amenable to analysis, prophase stages are much clearer than in the ladybirds. All three species have the same diploid count consisting of $9AA+Xy_p$.

Testes of these three species were freed from the investing fat body, fixed in 1 : 3 acetic-alcohol and stained in iron aceto-carmine, acetic orcein or by the Feulgen method. Aceto-carmine stains the nucleolus but the other methods do not.

2. OBSERVATIONS

The sex chromosomes are positively heteropycnotic and associated both in the pre-meiotic interphase nucleus and at early prophase. Their individuality cannot be recognised at this stage (plate, fig. 1). The autosomes are also characterised by heterochromatic segments in the vicinity of their centromeres but unlike the sex chromosomes these



(fig. 1)

(fig. 2)

TEXT-FIGS. 1 and 2.—*Adalia bipunctata*. First metaphase (fig. 1) and first anaphase (fig. 2) of meiosis. Note persistent nucleolus. ×4500 (Aceto-carmine).

are not regularly associated before the completion of zygotene. Indeed these centric H-segments, like those in *Blaberus discoidalis* (John and Lewis, 1959), are the last to pair. (plate, fig. 1). At pachytene the sex-bivalent stains less deeply than at earlier stages. It is still in advance of the distal euchromatic regions of the autosomes but is less condensed than their centric heterochromatin (plate, fig. 2). At this time the sex bivalent frequently assumes a signet-ring appearance and is associated with nucleolar material which we have failed to demonstrate at earlier stages (plate, figs. 3 and 4). It would appear therefore that there is a correlation between the change in pycnosity and the organisation of a nucleolus.

By metaphase the sex chromosomes and the autosomes are similar in their staining reaction. Preparations stained in acetic orcein or

feulgen show the typical parachute bivalent at this stage (plate, fig. 5). But with aceto-carmine the sex chromosomes are seen to be held together by a nucleolus (text-fig. 1 and plate, figs. 6-10). Usually this is found between the sex chromosomes; occasionally it is to one side, but this may be the effect of squashing.

At anaphase of the first meiotic division the y_p chromosome becomes detached from the nucleolus and this organelle moves to the same pole as the X chromosome (text-fig. 2 and plate, fig. 11). Like the persistent nucleolus of *Blaps* it does not disappear until first telophase.

We conclude that the formation of the Xy_p-bivalent is due initially to the characteristic stickiness of heterochromatin. But the maintenance and regular segregation of this bivalent is due to the formation of a nucleolus during prophase and its persistence until segregation is ensured.

It is now possible to understand why congression of the sex bivalent is not infrequently delayed (plate, fig. 7 and cf. Smith, 1952*a* and *b*) for the presence of a nucleolus must surely result in some degree of impedance to movement on the spindle.

3. DISCUSSION

(i) *Heterochromatic and nucleolar type sex-chromosome associations*

Reduction normally depends on bivalent formation which takes place in two stages:

(*a*) Specific pairing which leads to bivalent formation at zygotene.
(*b*) Chiasma formation which is responsible for maintaining the bivalent from diplotene to metaphase.

The second stage is conditional on the first but they are to some extent independently variable and the mechanism responsible for association at one stage is different from that at the other.

The inauguration of a sex-chromosome system on the other hand depends on a limitation of crossing-over and one of the commonest trends during the evolution of such a system is an increasing differentiation of the X and Y components. The differential segments extend at the expense of the pairing segments which may eventually be lost. In this event specific pairing and chiasma formation are both excluded.

Where the pairing segments are short the problem of ensuring zygotene association is increased. This is especially true when the differential segments are extensive for these, showing no tendency to specific pairing, are a " drag " to chromosome movement in the prophase nucleus. On *a priori* grounds therefore one might expect some means of facilitating specific pairing in such cases and a mechanism whose original mechanical function was merely to facilitate pairing could become modified during the course of evolution to maintain the metaphase association of completely differential sex chromosomes. What is subsidiary, incidental or even disadvantageous at one stage of evolution may prove indispensable at another.

Various investigators have often been, indeed still are, in disagreement concerning the relationship of particular sex chromosomes and especially with regard to the possibility of a chiasmate association between them. But for the moment we are concerned only with their initial association.

In this connection a particularly instructive series of relationships between the X and Y chromosomes exists in rodents where an RNA-containing sex vesicle is involved in their association. In *Microtus agrestis*, where the sex chromosomes are enormous compared with the autosomes, Sachs (1953) has concluded that the giant X and Y chromosomes are largely differential and that a chiasmate association forms in a small terminal pairing segment. Thus it appears that *Microtus* is an extreme example of the general case already considered. Now at leptotene the X and Y are both heteropycnotic but separate and each now organises its own nucleolar-like sex vesicle.—" The X and Y then move together until they are included in the one large vesicle at pachytene ". Thus, initial association is by the fusion of nucleoli. Leptotene proximity is favoured by the lagging of the X and Y at anaphase of the spermatogonial mitoses and the somatic pairing occasionally observed in the spermatogonia may have the same utility.

In the mouse too it appears that the positively heteropycnotic sex chromosomes exist as separate entities at leptotene, are embedded in a common sex vesicle at zygotene and are associated end-to-end at pachytene. Here, however, it is not clear whether the sex chromosomes associate before or after the vesicle forms (cf. Ohno, Kaplan and Kinosita, 1959*b*). In the rat on the other hand, the sex chromosomes appear to be associated with a common sex vesicle " from the beginning " (Ohno, Kaplan and Kinosita, 1956, 1958). Where heterochromasy and nucleolar formation go together their individual roles are not easily distinguished, but this is possible to some extent in the earwig *Forficula auricularia* where the sex chromosomes are completely differential.

In *Forficula* the sex chromosomes " come together at telophase of the last spermatogonial mitosis. They are fully condensed when they associate and it is highly improbable that their association leads to chiasma formation " (Callan, 1941). During the prophase of meiosis the sex chromosomes have a " covering of nucleolar material ", but in $2n=25$ chromosome-males, which are X_1X_2Y, a bivalent and univalent condition of the sex chromosomes at leptotene persists until first anaphase. In this species initial association depends on the characteristic " stickiness " of heterochromatin for which there is clearly no substitute.

There are many other cases where completely differential and heteropycnotic sex chromosomes, not involved in nucleolar formation, are associated non-specifically at first prophase. But, so far as we know, in all these cases the association always lapses prior to the segregation

of the X and Y components. We have personal experience of two of them, *Dicranocephalus agilis* (*Hemiptera: Heteroptera*) and *Tipula maxima* (*Diptera: Tipulidæ*), in both of which the first division is equational for the sex chromosomes. In the former (Lewis and Scudder, 1958) the sex chromosomes become negatively heteropycnotic at first metaphase. This change is not accompanied by the organisation of a visible nucleolus though material may be lost from the chromosomes. The X and Y then fall apart and behave as univalents dividing equationally at first anaphase. Prophase association leads to the X and Y auto-orientating side-by-side at first metaphase and this proximity doubtless facilitates the formation of a " pseudo-bivalent " at the second division of meiosis. The general sequence of events in *Tipula maxima* is similar, though considerably more complicated (John, 1957).

We would conclude therefore that the situation in our beetles is comparable to the above types in that initial association depends on the " stickiness " of heterochromatin, but that the organisation of a persistent nucleolus maintains this association and determines the reductional separation of the X and Y at first division. It is possible of course, that the real difference is not the presence or absence of a visible nucleolus but whether or not the materials which, in beetles, are organised into a nucleolus remain on the chromosomes or diffuse away. This brings us back to *Forficula*. If heterochromatic sex chromosomes not involved in nucleolar formation invariably fall apart the continued association of the sex chromosomes in *Forficula* may be associated with their nucleolar-type activity.

In the mouse the sex vesicle is the only nucleolar-like body present in the spermatocyte and it disappears at the end of diplotene (Ohno *et al.*, 1957, 1959*b*). Of *Microtus* Sachs says " the vesicle surrounding the sex chromosomes still persists at diplotene and diakinesis, but it has *in most cases* disappeared when the autosomes reach first metaphase". In the rat (Ohno *et al.*, 1956, 1958, 1959*a*) the spermatocytes also include autosomal nucleoli and although these disappear " before or during pachytene " the sex vesicle persists through diplotene: clearly it is different in some way. This appears to be the case in *Forficula* too (cf. figs. 1-3 of text-fig. 2, Callan, 1941). If negative heteropycnosity can indicate a loss of material it is significant that the sex chromosomes of *Forficula*, unlike those of *Dicranocephalus* and *Tipula*, do not show this property. It is possible therefore that they retain some part of the nucleolar material and that this accounts for their metaphase association.

The factors responsible for the association of the sex chromosomes in *Drosophila* have long been disputed. Here the paired regions include the nucleolar organisers and it has been suggested (Ohno *et al.*, 1957) that a remnant of the nucleolar material plays a role in maintaining the association of X and Y chromosomes at least until the end of diakinesis. Perhaps the diffusion of such materials to the chromosome ends can account for " terminal affinity ".

Finally persistent nucleoli of altered chemical composition have been described in many grasses. These do not stain in the post-prophase stages when aceto-carmine is used after the usual acid fixatives. They appear to be largely histone-containing, having lost their RNA (Brown and Emery, 1957). It may be therefore that the persistent nucleoli in beetles are distinctive only in the retention of this component beyond prophase.

(ii) *The situation in other Coleopterans*

A nucleolus associated with the sex chromosomes at prophase of meiosis has been described in the spermatocytes of a number of beetles, e.g., the curculionids *Sitophilus granarius*, *Sitophilus oryzæ* and *Sitonia lepidus* (Smith, 1952*a*) and the tenebrionids *Tribolium castaneum*, *Tenebrio molitor*, *Scotobates calceratus* and *Tribolium confusum* (Smith, 1952*b*); all but the last two of these have an Xy_p system. In these cases the nucleolus has not been described as present at metaphase but Smith invariably describes and figures the Xy_p bivalent as being negatively heteropycnotic at this stage. We have never found this condition. It is true that the nucleolus stains less deeply and a difference in interpretation may explain the disparity since small Xy_p bivalents are doubtless very difficult to analyse. We have no reason to doubt therefore that the nucleolus in other Xy_p coleopterans plays a role comparable with that in the species we have studied.

Smith (1956) has described one case where the nucleolus does persist until metaphase. This was in *Agriotes mancus*, an elaterid beetle with an XX/XO sex-chromosome mechanism. Clearly, the nucleolus here cannot play the role that it does in ladybirds: it would have lost its function with the loss of the y_p chromosome. Perhaps in this species we are dealing with a relatively recent evolution of the XO condition. The nucleolus probably lacks its mechanical function too in the comparatively recent neo-XX/XY system of *Tribolium confusum* but is indispensable in the phylogenetically older X*n*X*n*/X*n*Y systems like those in the genus *Blaps*. Thus, one of the conditions necessary for the successful evolution of the multiple sex-chromosome mechanism of *Blaps* was presumably present in the ancestral type.

The sex-chromosome-associated nucleolus may not be a " true nucleolus " in the sense that both sex chromosomes carry specific organisers responsible for organising nucleoli in physiologically active somatic cells. But rather a nucleolus with a special mechanical function needed and organised only in spermatocytes. This appears to hold for the sex vesicle in *Microtus* which has never been observed at mitosis (Sachs, 1953) and for the sex vesicle in *Rattus* which is produced in addition to autosomal nucleoli (Ohno *et al.*, 1959*a*). This view would help to overcome the difficulty to which we have already drawn attention (Lewis and John, 1957), of explaining how all the sex chromosomes are involved in the organisation of the nucleolus in the spermatocytes of *Blaps mucronata* which has an $X_1X_1X_2X_2X_3X_3/X_1X_2X_3Y$ mechanism.

Some evidence in support of this idea comes from the observation of secondary constrictions in the autosomes of *Adalia bipunctata* (text-fig. 3). Smith and Edgar (1954) on the other hand have described a satellited *y* chromosome in *Cicindela* but the evidence is not compelling. It may be of course that some coleopterans are like *Mus* and others like *Rattus* or *Forficula*.

(iii) *Some phylogenetic implications of the subsidiary devices*

It was argued above that where the homologous segments of the sex chromosomes were short, ensuring their regular pairing may present

Text-fig. 3.—*Coccinella septempunctata*. Spermatogonial metaphase. Note presence of a pair of satellited autosomes. ×4500 (Acetic orcein).

difficulty. It was further argued that other devices may facilitate initial contact in such cases and that one of these was the fusion of nucleolar-type organelles. While this may help to solve one problem it introduces another. Although it may help to bring chromosomes together it must surely impede the intimate pairing required for crossing over. Indeed Upcott (1936) has already shown that in both *Fritillaria* and *Eremurus* a nucleolus can reduce the frequency of chiasmata.

It may well be therefore that this device speeds up the very process that favoured its occurrence, namely the conversion of pairing into differential segments. As we have seen the differential segments of *Microtus* are extensive, and although the mouse has been the subject of intensive breeding experiments partial sex-linked determinants have yet to be found. Perhaps the nucleolus in these types will be ultimately called upon to serve the same function it now performs in ladybirds.

One would think that the same argument could be applied in those cases where the sex chromosomes are positively heteropycnotic when they associate. This too is the case in mammals and in at least some of these crossing over in precociously condensed segments has been claimed to occur. It is true that Ohno *et al.* (1959*b*) do describe a stage during the prophase of meiosis in the mouse where the sex chromosomes in the sex vesicle become negatively heteropycnotic and maximally extended (cf. John and Lewis, 1959). But it appears that the chromosomes are bipartite by now and that their terminal association has already been established by this time. Since the absence or infrequency of crossing over in precociously condensed segments has been recorded in so many other organisms the sex chromosomes of mammals are enigmatical in this respect and the situation requires clarification.

4. SUMMARY

1. In *Adalia bipunctata*, *Coccinella septempunctata* and *Tenebrio molitor* there is an Xy_p sex-bivalent in the male. This bivalent develops precociously as a result of the association of the positively heteropycnotic X and y_p chromosomes at pre-meiosis and is maintained until anaphase-I by the development during pachytene of a persistent nucleolus. This device enables the bivalent to achieve regular co-orientation in the absence of chiasmata though not infrequently the mechanical imposition of the persistent nucleolus leads to delayed congression. At first anaphase the y_p detaches itself from the nucleolus and the sex chromosomes segregate.

2. We are of the opinion that this mechanism probably operates in all XX/Xy_p systems and, since it necessarily loses its function in simplified XX/XO systems, the retention of a persistent nucleolus in at least one of these (*Agriotes mancus*) is suggestive of its recent ancestry. It would appear therefore that one of the factors responsible for the proper functioning of multiple X_nX_n/X_ny mechanisms like those of *Blaps* was antecedent to such systems.

3. We suggest that nucleolar material can play a supplementary or even indispensable role in the association of largely or completely differential sex chromosomes either early (rodents) or late (Coleopterans) in the meiotic cycle. Moreover the material may (Coleopterans) or may not (*Forficula*, *Drosophila?*) be organised into a visible nucleolus.

Acknowledgment.—We are grateful to Miss Anne Templeton for collecting the material used in this study.

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Plate

All figs. × 4500.

FIG. 1.—*Tenebrio molitor*, early zygotene. Heteropycnotic Xy_p association at ten o'clock. Note that six of the homologous autosomal centric H-segments have begun to pair (aceto-carmine).

FIG. 2.—*Tenebrio molitor*, pachytene. Xy_p association at twelve o'clock and now less condensed than the autosomal centric H-segments (aceto-carmine).

FIGS. 3 and 4.—*Tenebrio molitor*, pachytene. Development of the Xy_p associated nucleolus (aceto-carmine).

FIG. 5.—*Coccinella septempunctata*, metaphase-I. Typical parachute type sex bivalent (acetic orcein).

FIG. 6.—*Adalia bipunctata*, metaphase-I. Note the nucleolus between the X and y, chromosomes (aceto-carmine).

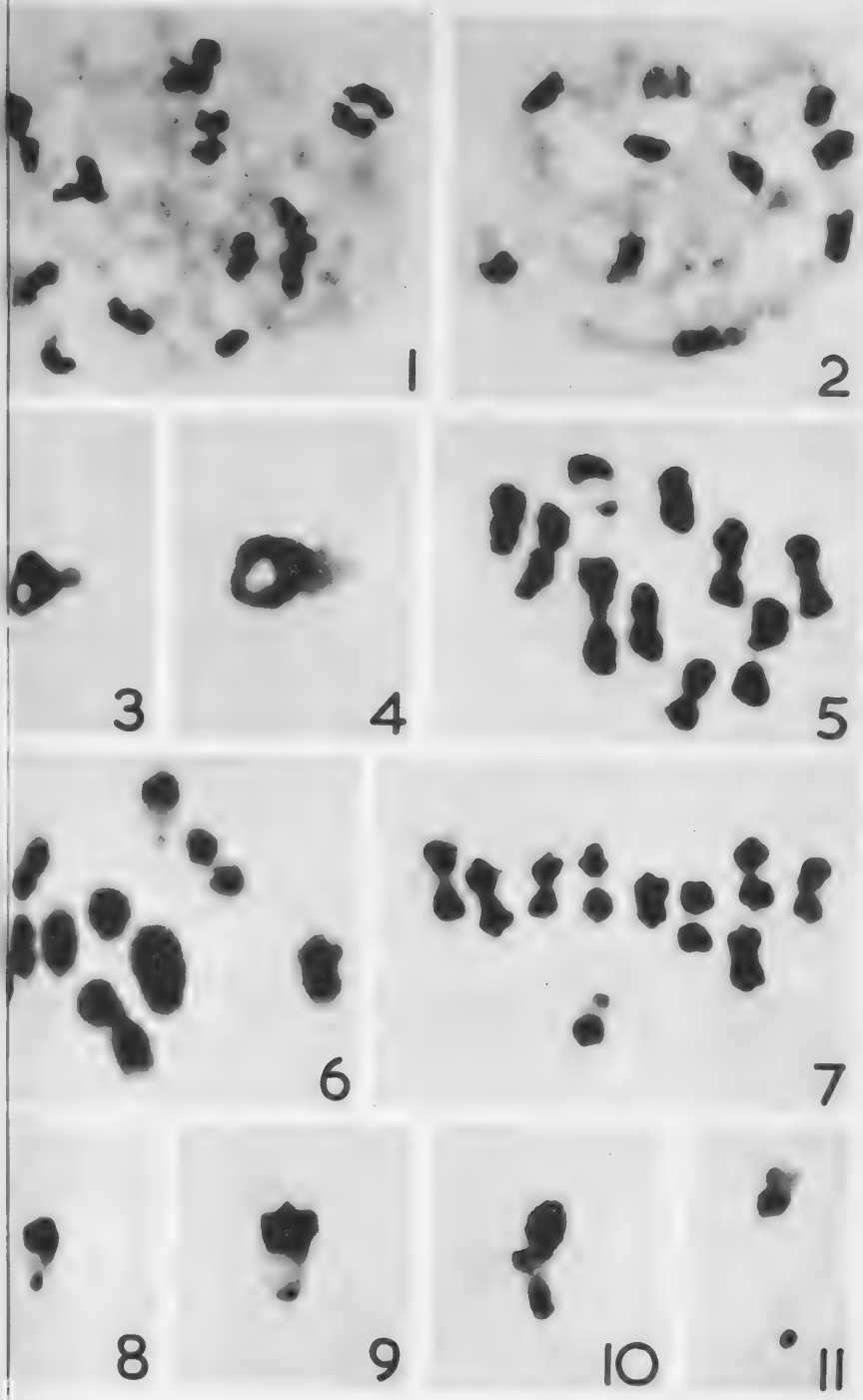
FIG. 7.—*Tenebrio molitor*, metaphase-I. Note Xy_p with nucleolus but uncongressed (aceto-carmine).

FIG. 8.—*Adalia bipunctata*. Structure of the Xy_p bivalent at metaphase-I (aceto-carmine).

FIG. 9.—*Tenebrio molitor*. Structure of the Xy_p bivalent at metaphase-I (aceto-carmine).

FIG. 10.—*Tenebrio molitor*. Structure of the Xy_p bivalent at early anaphase-I (aceto-carmine).

FIG. 11.—*Adalia bipunctata*. Segregation of the X and y_p chromosomes at anaphase-I. Note that the persistent nucleolus remains attached to the X (aceto-carmine).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Note added in proof

We have come across two additional relevant papers since our manuscript was submitted to press. First, N. M. STEVENS (1905), *Publ. Carneigie Instit. Wash.*, *36*, 3-71 described, under the term plasmosome, what is undoubtedly a neucleolar association between the X and *yp* chromosomes of the beetles *Odontata dorsalis*, *Trirhabda virgata* and *T. canadense*. Secondly, L. SACHS (1954), *Ann. Eugenics*, *18*, 255-261, claims that in man the X and Y chromosomes are held together within a sex vesicle which forms before the autosomes reach pachytene. Generally one such vesicle is formed but the X and Y may organise individual vesicles which subsequently fuse. Sachs also suggests that it is a remnant of the sex vesicle that holds the sex chromosomes together at Metaphase-I and that no chiasma formation occurs.

REVIEWS

THE FUTURE OF MAN: THE REITH LECTURES. By P. B. Medawar. London: Methuen. 1960. Pp. 128. 10s. 6d.

Here is a reprint of a series of lectures broadcast in English and other languages, and in many countries, in 1959. Twenty-four pages of explanatory notes have been added. The result constitutes a study of the methods, inferences and theories of genetics and of its applications to man. They are the work of a zoologist known to readers of *Heredity* for his success in applying genetic ideas in new fields, those of tissue transplantation and immunology. They therefore express a fresh and influential point of view on our problems.

Professor Medawar begins by explaining that what he is going to discuss is not strictly the Future of Man but rather how to foretell it. In order to do this we must analyse causes; we must not rely upon a purpose or a design. He goes on to discuss the growth of populations. Here he introduces the reader elegantly to certain tricks of scientific demonstration. He also recommends the method of cohort analysis which, he suggests, is likely to succeed where other methods have failed in predicting the future numbers of our population.

In his second lecture Medawar tells us what is now meant by " fitness ". It is the relative numerical share different individuals take in being the ancestors of future individuals. " The word ' fitness ', then "; he continues " has come to mean *net reproductive advantage*, and students of heredity, geneticists, do not deliberately use it in any other sense " (p. 28). It is a pity, if this is true, that the book gives no hint of where the lecturer has met this notion or expression, who invented it, and who has (in his opinion) succeeded in defining and using the idea and putting it into what he describes as " a central place in modern evolutionary thought ". It is a pity also that the lecturer does not attempt to use the idea himself. On the contrary he proceeds to discuss the selective effects of modern medicine and hygiene on man in terms that are ordinary and intelligible.

The use of drugs to cure disease, it has often been argued, will lead to a loss of fitness in human races on whose populations they are used. But, Medawar points out, the curing of malaria by drugs would lead to the extirpation of the present genetic means of restraint. The sickle-cell gene which is cruel and costly in its operation would slowly disappear. Unless, to be sure, its own effects could also be alleviated, a kind of problem which the lecturer discusses later.

At this point Professor Medawar breaks off to make a thrust at an unidentified antagonist. " It is simply not true," he declares, " to say that advances in medicine and hygiene must cause a genetical deterioration of mankind." (p. 33). Returning to his argument about disease the lecturer refers to a simpler case than malaria, that of phenylketonuria. This condition leads to imbecility. It represents an " inborn difference from other people, but it is no more a property of the genetic make-up *as a whole* than the inborn difference between people of blood-groups A and B." But who said it was? What difference is, or has ever been supposed to be, due to the

" genetic make-up " as a whole? On the other hand, what does a " genetic make-up " or genotype mean except as a whole?

The mistake of the lecturer arises from his failure to separate causes which are inferred analytically from consequences which are observed integrally. This mistake suggests a primary misunderstanding of genetics. The mistake however is at once turned to advantage. It provides the lecturer with a stick with which to belabour his imaginary antagonist, who is evidently a kind of bogeyman:

" To suppose, then, that congenital imbecility pointed to some general inborn inadequacy or degeneracy is nonsense—ignorant and cruel nonsense too. Our ambition should be to *cure* phenylketonuria, for it is an illusion to suppose that congenital afflictions are necessarily incurable; and if eventually we do cure phenylketonuria, we shall in no sense be conniving at a genetical degradation of mankind " (p. 34).

In no sense? But shall we not in some sense be arranging for a particular type of hereditary imbecile to breed? And will that have no consequences for mankind? Of mongolism however the author agrees that " it is not at all easy to see how it might be cured." But he concludes engagingly:

" When you come to think of it, all defects of the genetic constitution must have an accidental or unpremeditated or casually intrusive quality—' epiphenomenal ' is the word; for it is impossible, indeed self-contradictory, that any animal should have evolved into the possession of some complex and nicely balanced genetic make-up which rendered it unfit. It is this fact that justifies our always hoping to find a cure " (p. 34).

If by this paragraph the lecturer intends to convey a meaning, he must intend " epiphenomenal " to mean " secondary ". Thus mutation and mendelian segregation he is supposing to be secondary " qualities " of something, presumably of life, whose primary qualities he has not defined. Further the unfitness and consequent elimination of unfavourable products of mutation and segregation (such as is believed to occur by processes of natural selection) is " self-contradictory ". From this we are expected to conclude that mutation or natural selection is impossible or avoidable or remediable. Which is it to be?. It would be easier to conclude that the whole argument is irrelevant or meaningless or perhaps " epiphenomenal ".

The conclusion which the lecturer reached earlier however has a definite meaning. Advances in medicine and hygiene, he said, need not cause any genetical deterioration. Let us look at this proposition.

Primitive societies are made up of men and women who are able to do everything they need for their own survival and propagation. As families, if not as individuals, they are self-sufficient. Advanced societies on the other hand are differentiated into families and groups of families who are no longer self-sufficient. They are of mutually dependent kinds. Every step in civilisation has removed the specific pressure of natural selection from the populations enjoying its specific benefits. It has allowed the development of different groups or classes with wonderfully specialised aptitudes. But it has done so only at the expense of a loss of equally wonderful unspecialised aptitudes. Is this deterioration? Many people have said so and it is not easy to contradict them.

One of the most wonderful of these aptitudes is the development of modern medicine. With each successive invention of medicine our society becomes more dependent on medicine for the survival of its members. But

here something more serious arises. The tinker and the tailor are doing something *for* us. The physician and the surgeon are doing something *to* us. Formerly it mattered very little what they did. Today they are applying all the resources of science to alter the conditions of our survival and hence to change the course of our evolution. All the resources of science? All except those which are directly concerned with evolution; except, that is, genetics. Medawar tells us, or seems to tell us, that this is as it should be. We need not worry. Such things are, we may say, epiphenomenal.

His third lecture Professor Medawar devotes to giving some account of variation and of genetic systems. For him "genetic system", however, seems to carry no idea of its own; it seems to be a more respectable or more popular way of saying "genotype". He is not thinking of evolutionary series or interbreeding communities in nature. He is thinking still of individuals in the laboratory, individuals enjoying possibly some net reproductive advantages—or disadvantages. On this simple basis hybrid vigour is the property of an individual. As such it follows that hybrid vigour is "one of the mysteries of modern genetics" (p. 52).

Hybrid vigour becomes, nevertheless, the fulcrum of the argument in the next lecture. Here Professor Medawar has returned to the question of social conditions and has agreed that these (excluding perhaps medical or hygienic activities) all have genetic effects. Then, going back to the genetic system again, he observes or argues that the effect of inbreeding on "species like ours, genetically adapted to outbreeding, is invariably bad" (p. 59).

Thus, mystery or not, the lecturer is willing to make deductions from its supposed occurrence that would scare an ordinary geneticist. A world-wide species can be assumed to be "adapted to outbreeding." Genetic effects of its inbreeding can be assumed to be "invariably bad". Are they then perhaps worth avoiding by genetic methods? No. For we must not—according to the lecturer—be sure of any genetic conclusion. That would be the error of *geneticism*.

What is geneticism? Geneticists will want to know. It is "the application to human affairs (or indeed to livestock breeding or natural selection or evolution) of a genetic knowledge or understanding which is assumed to be very much greater than it really is" (pp. 61-62). It seems that this error is one the author himself once, a long time ago, fell into. For, he confesses, "twenty years ago it all seemed easy". The reason is now plain, however. "The trouble is that the whole way in which we think about genetics has grown up round these somewhat unusual cases"—cases of major gene mutation (p. 66).

Here the lecturer, it seems, is speaking for genetics. He is telling the world about the history of the subject. But has not a great deal of genetics grown up round quite different kinds of observation? Opposed to the analytical but static idea of the gene, dynamic and integral ideas have grown up. They have often been connected with adaptation and balance: sex determination and the complexes of *Œnothera* both connected with special segments of chromosomes; polymorphism with its evolving and adaptive—and usually unanalysable—super-genes; chromosome breakages structural hybrids and polyploids; the distinction between two kinds of chromosomes and several sorts of genes; an evolution of genes as well as of genetic systems; various kinds of sexual, subsexual and parasexual reproduction; these notions clarifying and explaining and connecting with one

another. Such ideas of the genetic material in nature have been growing up for about fifty years side by side with our knowledge of the gene in breeding experiments. The two sources of understanding have always been complementary. The insufficiency of each and the problem of their integration has been present to the minds of many geneticists. The elementary academic gene which Professor Medawar complains about was already a museum specimen when he met it and with some enquiry he might have noticed its condition and reputation twenty years ago.

The argument continues with the statement of a " discovery of Newtonian stature," namely that " the genetic structure of a randomly interbreeding population remains constant from generation to generation except in so far as some impressed force (like natural selection) is brought to bear upon it " (p. 64).

If the lecturer had paused to think, he would have hesitated to make the Newtonian analogy. He would have remembered the effects of disuse. He might have recalled the idea of stabilising selection. He might even have enquired about the stability of chromosome numbers maintained by selection in the individual and in the species. And, having thought so long, he might have seen somewhat differently, amongst other things, the future of man.

Professor Medawar, however, now returns to his bogeyman. He prepares to take up the weapons of polygenic inheritance in his struggle. Polygenic people, he claims, would be, and indeed already are, on his side against the enemy:

" They are the last people in the world to make cock-sure predictions about the consequences of selection—least of all about selection as it affects the welfare and destiny of man." Without stopping to give reasons the lecturer continues: " It is not true that we now know how to control our own evolution—if by ' control ' is meant directing it towards a predetermined goal. We are *not* entitled by our present knowledge to put a genetical construction upon the rise and fall of nations. We do *not* understand the inheritance of differences of temperament or character; all we do know about the matter is what we have learnt from the evolution of tameness and docility in domesticated animals; that some such characters are under some kind of genetical control " (pp. 66-67).

In the sense defined, however, we can control evolution in every other sexually reproducing organism. And without needing more genetics than was known to the author of the Book of Genesis. Then why not in man? Perhaps it is that we ought not to control it? But that is a different question. As for the inheritance of temperament and character and the genetic interpretation of social changes, whom is the lecturer confuting? What bogey is he now denouncing? He might be challenging Darwin. Is he rebutting chapter 12 of *Animals and Plants*? Or is he quarrelling with opinions on behaviour and evolution expressed in a recent symposium? Or has he just not read the evidence, ancient or modern? We cannot tell.

Professor Medawar turns now to the particular problem of birth control. Unlike other new aspects of medicine and hygiene, birth control, he feels, could have unfavourable evolutionary effects. But he treats birth control without reference to population numbers just as in his first lecture he treats population numbers without reference to birth control. May we now look at them together?

A big selective change arose from the introduction of birth control.

Previously children had been born to parents merely in accordance with their ability to beget and bear them. Now they were born to parents in accordance with their willingness. Thus, for the first time in evolution, parents who did not want children, or want so many of them, were selectively disfavoured. Conversely a selection began which favoured specifically the property of wanting children. We should therefore expect a sag in the birth rate; and after the sag a recovery. As indeed seems to have happened. For Professor Medawar this would be perhaps a worthless speculation. A piece of geneticism, he might say.

The fifth lecture gives us an account of the relations of fertility and intelligence. The lecturer agrees that both are subject to heredity and the two as a rule are negatively correlated which could have an unfortunate effect. Following this argument, however, is a statement that seems to contradict it. " There is no need to assume that professional men are innately more intelligent than labourers " (p. 84). But the intelligence tests which the lecturer accepts for his argument have indicated that professional men are innately more intelligent than labourers. Moreover, is this not what we might expect? For selection is one of the main tasks of universities. And its operation has long been directed to selecting from the general population the most intelligent men to enter the professions.

The last lecture attempts to consider man's capacity for the development of culture by the activity of his brain. " The attempt," Medawar begins, " must be based on hard thinking . . . based upon particulars, as opposed to that which finds its outlet in the mopings or exaltations of poetistic prose." For some time the lecturer has been using the expression " genetical instruction." This is a valid metaphor and avoids the use of the distasteful (and perhaps geneticistic) phrase " genetic determination." But when he comes to the brain he has to invert the metaphor. The chromosomes carry instruction. The brain now carries heredity. They are both " biological heredity".

Can one see what is meant? Probably, if one carefully translates these phrases into ordinary language. But to most listeners, perhaps to all, the upside-downness would be confusing. They would perhaps be relieved when the lecturer went on to say that the " similarities and correspondences between the two systems of biological heredity " were almost of no importance to us (p. 97). Probably they would have been happier not to have had instruction called heredity or heredity called instruction but to keep to the old-fashioned meanings of these popular but usefully contrasted words.

The lecturer's terminology, however, seems to be a necessary part of his argument. For by using it he convinces himself (p. 98) and wishes to convince us (p. 99) that " our newer style of evolution is Lamarckian in nature ". Thus it seems to him, as it has to many others before, that we can cut the Gordian knot of our social problems with one stroke. To the reviewer this one stroke seems to tie them all up in utter entanglement. It is an entanglement which the " profound truth " that " nature does not know best " fails to disentangle. For we have all long believed—have we not?—rightly or wrongly, and in our different ways, that we knew better than nature.

The lecturer's conclusion is a last attack on his assembled bogeys:

" The conception I have just outlined is, I think, a liberating conception. It means that we can jettison all reasoning based upon the idea that changes

in society happen in the style and under the pressures of ordinary genetic evolution; abandon any idea that the direction of social change is governed by laws other than laws which have at some time been the subject of human decisions or acts of mind. That competition between one man and another is a necessary part of the texture of society; that societies are organisms which grow and must inevitably die; that division of labour within a society is akin to what we can see in colonies of insects; that the laws of genetics have an overriding authority; that social evolution has a direction forcibly imposed upon it by agencies beyond man's control—all these are biological judgments; but I do assure you, bad judgments based upon a bad biology " (p. 99).

There is a danger that this book will not be widely read or discussed. The lay reader will enjoy its fervour but will not understand much beyond its moral judgments. The general scientist will recognise the confusion of ideas and suspect the unsupported assertions of what is good or bad or merely hopeful. The geneticist will think the lectures shallow. So they are. But their shallowness is obvious only because the questions Medawar poses are deep ones and part of a subject he does not deeply understand. They are part of genetics. If others, therefore, may disregard his replies the geneticist should give them some consideration.

C. D. Darlington.

MICROBIAL GENETICS. Society for General Microbiology Symposium No. 10. Cambridge Univ. Press. Pp. 300. 42s.

The Society for General Microbiology and the Editors, Drs Hayes and Clowes, are to be congratulated for the publication of a volume which covers most aspects of a very wide field and yet maintains almost throughout a high standard. Reading between the lines of the Editors' preface and of the Introduction (by Stocker) one might venture the guess that among the aims of the Symposium there was that of " educating " the non-initiated microbiologist. There is undoubtedly a substantial proportion of microbiologists not yet aware of the changes brought into their own field by the genetical approach. Conspicuously absent up to about 1940, since then it has revolutionised microbiology to such an extent that the fields impervious to it are dead or moribund, while those which it has permeated have raised microbiology to a central position in biology.

If this guess is correct, outstanding as the Symposium was in every other respect, it failed in this missionary aim. Fortunately few of the authors wasted time—and not successfully at that—in attempts to explain what a willing microbiologist can learn from an elementary textbook of genetics and an unwilling one will not learn anyhow.

It is impossible to review here in detail the fourteen papers of the Symposium—most of which are very good. I shall be able only to pick a few which struck me as important, though the others are not necessarily less so. Hayes's paper " The Bacterial Chromosome " is an excellent critical review of the present knowledge on the genetics of bacteria both from the cytological and the genetical angles. It is particularly effective in summarising the evidence for a circular linkage group. The paper by Jacob, Schæffer and Wollman " Episomic Elements in Bacteria " puts forward in a comprehensive way the concept of " episomes " proposed by

Jacob and Wollman two years ago. These are defined as dispensable genetic elements which, when present in a cell, may exist in two alternative states, either as autonomous structures replicating independently of the chromosome, or integrated in some unknown way with a particular segment of the chromosome, and replicating in step with it. Three examples, answering this description, are well established in bacteria: lysogenising viruses, sex factors and colicinogenic factors. Others—like the sporogenic factors—are suspected. Episomes are distinct from both purely chromosomal and purely cytoplasmic genetic elements, because they can alternate between integrated and autonomous states, and when in the latter state they are transmissible by infection. An interesting variety of consequences in nucleo-cytoplasmic interactions are to be expected according to whether an episome is absent, autonomous or integrated, and whether it is subject or not to the genetically built-in mechanisms of control which the group at the Institut Pasteur has so brilliantly begun to recognise.

A remarkable paper by Pritchard disposes very effectively—on the basis of original results with *Aspergillus*—of a number of myths which have recently fogged the study of recombination: e.g. a basic difference between intra- and inter-genic recombination; the idea that linearity within genes is not continuous with linearity between them, or that a mechanism of recombination by switch in copy-choice leads necessarily to non-reciprocal products, etc. Pritchard gives a comprehensive account of his model of recombination by discontinuous " effective pairing " and of the reasons why recombination in organisms with a microscopically observable chromosome apparatus is compatible with, but not fully explained by, a process of copy-choice at the time of replication: this implies that recombination occurs well before pachytene.

The paper by Garen on the genetic control of alkaline phosphatase in *E. coli* gives an exciting example of work—of a kind in progress in four laboratories in the world—which will soon give us the first pair of pictures; a map of a gene in terms of a sequence of mutational sites, and the map of the polypeptide chain determined by that gene in terms of a sequence of amino-acids. The race is on between four systems: alkaline phosphatase and tryptophane desmolase in *E. coli*, lysozyme and " head protein " in bacteriophage. There is so much expectation that the answer should be one of some sort of co-linearity that what should be hailed as one of the most spectacular feats of biology may well come, if and when it comes, as an anticlimax.

In relation to problems of this kind the paper by Gierer, on ribonucleic acid as genetic material in viruses, is perhaps somewhat disappointing. The demonstration by the Tübingen group, and foremost by Gierer, that the RNA moiety, and not the protein, of tobacco mosaic virus carries genetic specificity was no mean achievement. The present paper shows how much farther in detail the work has gone, particularly as a consequence of the demonstration that de-amination of individual nucleotides by treating the RNA with nitrous acid may be mutagenic. To me, the disappointment arises from the fact that material like this, so wonderful from every other aspect, has not been harnessed to fine genetic analysis, or indeed to any kind of genetic analysis. Unless some completely unexpected approach develops, it will not be possible in this system to correlate sequence of bases in the virus RNA, sequence of amino-acids in the virus protein and

in the host enzymes induced by virus infection, and sequence of mutational sites in the virus genome.

A very brilliant paper by Maaløe gives a theoretical sketch of how a cell may adjust the sizes and activities of its synthetic systems to the nutritional content of the medium. The value of this paper, especially for people, like myself, not versed in the subject, lies in the way it brings together information from different fields. A major recent contribution to biochemical genetics is the discovery by Cohen and Jacob of what we could call the principle of " two (or more) genes-one-enzyme ". For some enzymes there seems to be at least two kinds of genes: one kind determines the aminoacid sequence (or sequences) of the polypeptide (or polypeptides) which make up the enzyme: the other kind determines the formation of a specific " repressor ", apparently not protein, which checks the synthesis of the enzyme unless inactivated by specific small molecules of internal or external origin. This is what underlies the induction of certain enzymes by a specific substrate and the immediate repression of the synthesis of a whole series of enzymes in the exogenous presence of their metabolic end product. Maaløe discusses how the flow of information from the nucleus to metabolic end products and from them back to the nucleus determines the adjustments which lead to a definite growth rate and cell composition.

Other papers of the Symposium are well worth reading. Kellenberger gives an account of nuclear structure based mainly on electron microscopy. Clowes provides a useful review of fine genetic analysis by means of transduction, and Esther Lederberg gives the details of the joint biochemical and genetic analysis of galactose metabolism in *E. coli*. Harriet Ephrussi-Taylor develops some important ideas on the functions of DNA from transformation studies: unfortunately the presentation is so difficult to follow that I, for one, cannot understand more than a fraction of the paper. The same criticism applies to a probably very valuable paper by Brown on " DNA and Specific Protein Synthesis ". Catcheside's paper shows how interesting and widespread is inter-allelic complementation.

The only dissonant note in the Symposium is Danielli's paper on " Inheritance in *Amœba*, studied by nuclear transfer ". One has only to compare this paper with that of Jacob and colleagues and that of Maaløe, to see that in choosing a genetically quite unanalysed organism like *Amœba* for studying problems of nucleus-cytoplasm relations, he has chosen to bite off more than can be chewed.

G. Pontecorvo.

BIOCHEMISTRY OF HUMAN GENETICS. Ciba Foundation Symposium. Ed. by G. E. W. Wolstenholme and C. M. O'Connor. London: Churchill. 1959. Pp. 347. 50s.

The application of biochemical techniques to the study of human variation during the past decade has produced results of interest not only for medicine and anthropology but also for fundamental genetics. The main lines of development were covered in papers presented at the Ciba Foundation Symposium held in Naples in May 1959.

Hunt and Ingram reviewed their work suggesting that several abnormal human hæmoglobin types differ from the normal adult type by single amino-acid substitutions in one or the other half-molecule. Similar substitutions are often found when proteins in different animals are compared, and

it is becoming clear that this is the commonest way in which mutations affect the protein products of genes. The normal adult human hæmoglobin molecule consists of two pairs of unlike polypeptide chains, termed α and β. It appears that these are under independent genetic control and either the α or the β chain can be altered by mutation. Itano and his colleagues outlined experiments in which mixtures of hæmoglobins abnormal in respect of α and β chains were dissociated under mildly acidic conditions and recombined to give normal and doubly abnormal forms. Similar random combinations of protein subunits occur naturally and probably explain phenomena such as gene complementation in *Neurospora*.

Genetic variations in human serum proteins were described by Connell and Smithies. The several forms of transferring are analogous to the hæmoglobins, but the haptoglobins are somewhat different, in that one form of the protein polymerises and in the heterozygote gives products which appear to be qualitatively distinct from those in either homozygote. However, as Allison and Smithies have pointed out, this is probably due to co-polymerisation of the primary products of the two genes. The distribution of the plasma protein variants in some populations was outlined by Harris and colleagues, and genetic differences in human gamma globulin types, recognised serologically, described by Grubb.

Morgan and Watkins reviewed advances in the chemistry of human blood group substances made in their laboratory and Kabat's in the United States. The sugar and amino-sugar end-groups defining A, B, H and Le^a specificity have been defined. Ceppellini contributed a valuable synthesis of a difficult topic, the inheritance of the A, B and Lewis factors and their relationship to secretion.

Inherited deficiencies of enzyme activity are known to be responsible for a number of rare metabolic disorders, e.g. galactose uridyl transferase for galactosæmia, as outlined by Kalckar at the symposium. The abnormal form of plasma pseudocholinestrase in human subjects, described by Kalow, is rather more common and is of medical importance because affected individuals cannot tolerate normal doses of muscle relaxant drugs. Other deficiencies are remarkably common, including glucose-6-phosphate dehydrogenase in red blood cells of Mediterranean, African and Oriental males. This condition, and its relationship to the disease "favism", produced in affected subjects by ingestion of broad beans, and also to hæmolysis on exposure to a variety of drugs, was discussed by Childs and Zinkham. Kalmus reviewed work on inheritance of sense organ disabilities, and Brenner contributed a general account of the part played by proteins in gene mediation.

In general, this volume is well produced and provides a convenient summary of work in the field up to early 1959. It also helps to show that despite the obvious difficulties of studying inheritance in man much valuable progress has been made. There is still a future for biochemical geneticists who recognise the proper study of mankind.

A. C. Allison.

STATISTISCHE METHODEN DER POPULATIONSGENETIK. By H. L. Le Roy. Basel: Birkhäuser. 1960. Pp. 397. sFr. 67.50. (c. 112s.).

The term "Population Genetics" has a disconcerting habit of changing its meaning slightly with each author who uses it in his title. Here the

meaning is not that of Li (and consequently there is little overlap with the contents of Li's book), but has returned more nearly to that of Lerner, *i.e.* the genetics of continuously varying characters. Professor Le Roy is concerned primarily with the mathematical content and hardly at all with the application or experimental study of the subject. He describes with indefatigable thoroughness the mathematical reasoning underlying the genetics of continuous variation. Indeed, English readers are likely to find the thoroughness more than a little overpowering. Nevertheless, for the determined student, anxious to follow through every detail of the algebra, the book will serve as an authoritative text for a long time to come.

There are four main sections. The first, after a short and confusing explanation of non-genetic variation, develops the algebraic formulation of mean and variance in terms of gene frequency, average effect, dominance and epistatic deviations, and correlation and interaction between genotype and environment. An enlightening series of photographs of three-dimensional models illustrates the effect of gene frequency on the main components of genetic variance arising from two loci segregating simultaneously. The second section deals with the theoretical basis of correlation between relatives, and the third with the estimation of the genetic and environmental components of variance from analyses of variance. Particularly useful here are the explanations, not readily accessible elsewhere, of how to deal with unequal sub-class numbers in analyses of variance, and of how to calculate the standard errors of variance components and of the heritability estimated from them.

The fourth section deals with artificial selection and shows how the expected response is influenced by the various factors governing it, including the use made of information from relatives and of correlated characters. The progress expected from recurrent and reciprocal recurrent selection is also described. This section on selection, which comprises one third of the book, suffers more than the others from the lack of any but the briefest references to experimental or practical results. It describes what ought to happen without considering at all what actually does happen when artificial selection is applied. The author explains in his preface that space forbade the inclusion of experimental results, but it would surely have been wise to give some indication that selection responses do not always behave according to expectation, and that there are complications as yet unresolved.

The outstanding omission from the contents of the book is any account of inbreeding, which is mentioned only briefly in connection with an explanation of Fisher's " average excess ". Another regrettable omission is that of a subject index. The convenience to users of the book would surely have justified the additional pages required. In place of an index there is an ingenious but confusing table of contents arranged under a two-way classification of subjects. The list of references is arranged in two sections, a short list (3 pages) of works cited in the text, and a long list (28 pages) of other references extending up to 1958, grouped according to the subject. This is probably the most extensive published bibliography of the subject and will be of great value. The book is extremely well produced, the mathematics and tabular material being set out with great elegance. The very high price is not surprising in view of the fact that about 70 per cent. of the book consists of algebra, tables, or figures.

D. S. Falconer.

THE ORGANISATION OF CELLS AND OTHER ORGANISMS. L. E. R. Picken. Oxford University Press. 1960. Pp. 629. 84s.

Since the publication of Wilson's *Cell* in its 3rd edition of 1925 authors who have attempted to survey the field of cytology have usually satisfied themselves with one part or with one aspect. In the present work Laurence Picken may seem to have attempted to encompass the whole subject. Indeed Dr Picken (who is of the Zoology Department in the University of Cambridge) has gone boldly and properly beyond the classical compass of cytology by including his views on bacteria and viruses. The word *organisation*, however, defines his limit. He is thinking of how the thing works and he is confining himself to examining how the observation of structure and the evidence of working help us to understand both.

In effect, however, Dr Picken does not go so far as this. For if he did he would have to include much more of the evidence of genetics than he does. In practice the limit he reaches is obviously the limit of his own interests. These are wide enough. In a sense they are too wide for the comfort of the reader. For he considers with some intricacy of detail the most recent evidence of the chemistry of chromosomes and the electron microscopy of fibres and cytoplasmic particles. And at the same time he gives us a range of valuable opinions on the history and philosophy of his subject, especially animal cytology and embryology. Inevitably he fails to maintain the same level throughout. His genetical references are not at all up-to-date. A good deal of valuable detail may also be lost simply through the great size of the book.

While the whole of this book will interest very few readers, parts of it, whether text, plates, bibliography or the highly analytical index will interest very many. But what is perhaps most important about it is its scope and purpose. Dr Picken wants to show, what others have feared to show, that the study of the different parts of the cells, whether they belong to plants, animals or bacteria, and whether they are concerned with heredity, development or infection, hang together and can be discussed together. He has manifestly succeeded. And in doing so he has given something of a new look to cytology.

C. D. Darlington.

A PROPOSED STANDARD OF NOMENCLATURE OF HUMAN CHROMOSOMES. By a Human Chromosome Study Group. Supplement to Cerebral Palsy Bulletin, Vol. 2, No. 3. 1960. Pp. 9. 5s. or $1.

If Voltaire is to be believed "*common sense is not so common*". It is encouraging therefore to find that the principal students of human karyology recently had the good sense, and apparently the capital, to meet together in Denver and recommend a provisional standard system of nomenclature for the chromosomes of man. The results of their recommendations are to be found in this clear and concise report which will surely be indispensable to all workers in this field. It should also bring a measure of relief to those of us who in the four years during which human chromosomes have become a vital component of the "*materia medica*" have had to reconcile for ourselves the six distinct systems which have been in vogue in different laboratories. The report is illustrated by two excellent photographs and two instructive tables. The only important omission seems to be in table 2 where no indication is given either of the total number of cells on which measurements were made or the number of individuals from which these cells were taken.

That the association between medicine and money is more causal than casual has long been apparent to those of us not so comfortably endowed. In the present instance this association is reflected not only in the fact that such an expensive conference could be justified in terms of such restricted subject matter, but also in the relatively high cost of such a small publication. Perhaps the glossy cover and the seven empty pages for " notes " are intended to reduce this discrepancy.

B. JOHN.

REPORT OF THE VIth INTERNATIONAL BLOOD GROUP CONGRESS IN MUNICH, 21-23 August, 1959. Chairman: Professor A. Meyn. Tierzuchtfrorschung e.k., Instititute für Blutgruppenforschung, München 15, Haydnstrasse 11. No price stated.

The sixth international blood group congress convening in Munich was concerned with the genetical and serological aspects of blood group work in cattle, swine, chickens and dogs as related to practical production problems, parentage identification and surgery.

The relations between blood group genes and certain milk factors were demonstrated by Rendel (Uppsala), Neimann-Sorensen (Copenhagen), Tolle (Göttingen), Andresen *et al.* (Copenhagen). Rendel reported a definite relationship between the B-locus and fat percentage of milk. Cows carrying the allele $B^{BO_1Y_1D'}$ produced milk with approximately 0·16 units higher fat percentage. On the other hand he found no consistent influence of any blood group genes on the total butterfat yield or the heart girth measurements. Andresen *et al.* report a positive effect on butterfat due to the BO_1Y_1D' group but a negative effect on total milk yield. They report a significant reduction in milk yield for the QOJ " K " group. They conclude that the variability of the blood groups within and between breeds may be the result of the differential selection value of such pleiotropic effects on butterfat percentage and milk production. Work on the serology and genetics of blood groups in swine and dogs is reported also.

Gilmour, in an extensive review of blood groups in chickens, reports six series of red cell antigens identified by agglutinating iso-immune sera. Each of these is determined by a separate chromosomal region. In the case of the antigens of the A-E series, there is evidence of two closely linked loci. The question of transplantation antigens in connection with the phenomenon of immunological tolerance is reviewed. (Matousek, Czechoslovakia, also reports on this subject in a separate paper.) The general conclusion was that leucocytes and a variety of other tissue cells possess the antigens responsible for transplantation immunity against skin and other tissue homografts, while the red cells do not. Finally he mentions the application of the knowledge about chicken antigens to commercial poultry breeding. Apparently, marked differences occur in the reproduction performance and viability of the different B locus heterozygotes among progeny of crosses of inbred lines.

The reports of the individual blood group laboratories participating in the congress present evidence of progress in blood group serology and the genetics of animal blood groups.

The congress is notable for initiating an international standardisation of animal blood typing reagents and for drawing up and adopting rules for the selection, storage and use of blood typing reagents in cattle.

J. GRAFIUS.

GENETICAL SOCIETY OF GREAT BRITAIN

ABSTRACTS of Papers read at the HUNDRED AND THIRTY-FOURTH MEETING of the Society held on 11th and 12th NOVEMBER 1960, at the UNIVERSITY COLLEGE, LONDON

ANEUPLOIDY IN YEAST

B. S. COX
Botany School, University of Oxford

In 1958, Bevan reported that a strain of yeast, heterozygous for an adenine requirement which confers a red colour when present in the homozygous condition, produced many colonies with red sectors when it was plated on complete nutrient agar. It has been possible to demonstrate, through the existence of partial heterozygosity in the haploid offspring, that this diploid was a tetrasomic of constitution 2n+2=AD/ad/ad/adl. It has been shown that the frequency of sectoring of this and other aneuploids can be very much higher than that of normal heterozygous diploids, but that the frequency varies between one aneuploid and another, and even between different sub-cultures of the same aneuploid strain. It is suggested that fluctuations in the chromosome complement are responsible both for the appearance of the red sectors themselves and also for some of the variation in the frequency with which they appear. Experiments are also described which suggest that some of this variation may be due to cytoplasmic factors.

THE ASSOCIATION OF CHROMOSOMES IN HAPLOID COTTON

G. KIMBER
Plant Breeding Institute, Cambridge

The chromosomes of the allotetraploid cottons derived from the Asiatic diploids (A genome) are larger than those derived from the American diploids (D genome). Analysis of meiosis in polyhaploids of *Gossypium barbadense* reveals that whilst there is no bivalent formation there is a frequent association of chromosomes of dissimilar size and a less frequent association of chromosomes of a similar size.

The evidence from polyhaploids and from certain interspecific hybrids points to the possibility of genetic control of intergenomic chromosome pairing in the allotetraploid cottons, similar to that in *Triticum æstivum*. Since earlier data on *Nicotiana tabacum* hybrids can be similarly interpreted, the genetic control of the cytologically diploid behaviour of allopolyploids may be a frequent occurrence.

CHANGES IN FERTILITY AND VIGOUR ASSOCIATED WITH SELECTION FOR EAR EMERGENCE IN *LOLIUM*

J. P. COOPER
Welsh Plant Breeding Station, Aberystwyth

Selection for early and late ear emergence in Irish and Kent ryegrass has produced rapid and continued response for six generations. Although the selection differential is similar in both directions, the realised heritability is always higher in the late lines. Both the selection differential and the heritability are greater in Kent than in Irish, possibly because of less stringent agronomic selection in the past. Back-selection, which was started in each line after the first generation, indicates that considerable potential genetic variation can be carried within four foundation plants.

Male and female fertility have declined in all lines by the 6th generation, and in some cases severely reduced the possible selection differential. The variation in fertility both *between* and *within* plants was also increased in the selection lines. Although the mating system employed results in slight inbreeding no regular depression in such criteria of vigour as leaf size, tiller number or dry weight per seedling could be detected, nor was there any regular correlated response in these characters.

GENETIC AND ONTOGENETIC VARIATION IN THE GROWING FORM OF THE DOMESTIC FOWL

A. G. COCK
A.R.C. Poultry Research Centre, Edinburgh, 9

In a population of mixed breed origin, selection in opposite directions for relative length of shank (tarsometatarsus) has been carried out, using $\log y_{10} - 0{\cdot}4 \log x_{10}$ as an index, where y_{10} and x_{10} are shank length and body weight at 10 weeks of age. Two generations of selection have produced a divergence of 0·031 (equivalent to 7·5 per cent. difference in relative shank length) between the selected lines, the realised heritability being 0·55. A correlated response (in the opposite direction) in relative shank width has occurred.

The ontogenesis of these differences in form will be discussed. It will be shown that : (i) over the period 2-10 weeks the relative growth-rate (k) of $\log y$ with respect to $\log x$ is approximately constant within individuals, with a mean value close to 0·4. (ii) In the initial population, variation in k between individuals is highly significant, but does not account for all the variation in $\log y$ at a fixed $\log x$ (the regression lines do not pass, within the limits of error, through any single point). (iii) Although the selection applied has been partly directed towards changing k, in the first generation at least, no divergence in k has occurred.

THE SYNDROME OF CONGENITAL DEAFNESS WITH ABNORMAL ELECTROCARDIOGRAM

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M.R.C., Population Genetics Research Unit, Oxford

P. FROGGATT
Department of Social Medicine, Queen's University, Belfast

One type of recessively inherited congenital deafness is found to be associated with qualitative and unique abnormalities in the electrocardiogram. These have not been found to be correlated with any identifiable biochemical or pathological lesion either in life or post-mortem. The syndrome may be associated with an undue predisposition to fainting attacks in childhood which have in some cases terminated fatally.

The clinical and genetical features of this syndrome are discussed in relation to five families found during a survey of deaf school-children.

CHROMOSOME NUMBER VARIATION WITHIN A *RUBUS* PLANT

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One abnormal stunted seedling occurred in the thornless segregants of an F2 progeny from a cross between Merton Thornless (*R. craniensis*) and Himalaya Giant (*R. procerus*). Cytological investigations showed this seedling to be unstable in somatic chromosome number (root-tips and stipules), with a range of $2n=9$ to 46; the mode was at the pentaploid level ($2n=35$). The range in chromosome numbers decreased, while the actual numbers of cells with 35 chromosomes increased as the

plant aged. Both parents and sibs were constant tetraploids of 2n=28. Tests showed that viruses did not cause the instability.

Within root-tips for the aneuploid series there was a correlation between cell size and chromosome number of $r=0{\cdot}65$, significant at the 5 per cent. level. It is suggested that because *Rubus* chromosomes are more or less alike, introduction or removal of individual chromosomes into a cell is comparable to introducing or removing equivalent amounts of DNA to give proportional changes in cell size. It has been suggested by other workers that the amount of DNA affects cell size in a strictly polyploid series in rats.

Somatic chromosome number variation in the vegetative system may be another facet of the reproductive versatility in the genus *Rubus*.

A LATENT GENE CONTROLLING DDT SENSITIVITY IN *HOREDUM*

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Welsh Plant Breeding Station, Aberystwyth

Barley gives a distinct reaction to spraying with DDT. Certain varieties are resistant and do not manifest any external symptoms of damage, while susceptible varieties suffer severe chlorosis. Resistance to DDT in cultivated barley (*H. vulgare*) is controlled by a single recessive gene (*ddt*) and it appears to be distributed at random through the commercial varieties. Populations of wild barley (*H. spontaneum*) have also revealed similar variability in their reaction to DDT spray, which indicates that the gene does not appear to have had any selective advantage in the evolution of the barley crop.

Under severe DDT treatment, susceptible genotypes of barley are eliminated while resistant genotypes remain unharmed. The possibility of linking this phytocidal action with male sterility (*ms*) has led to the proposal that a system of breeding hybrid barley on a field scale could be developed.

The genetic control for reaction to DDT for barley leads to a consideration of the possible existence of similar control systems in other crops and also for other chemicals.

GENETIC RECOMBINATION IN *SALMONELLA TYPHIMURIUM* MEDIATED BY CERTAIN COLICINE FACTORS

H. OZEKI, S. HOWARTH, S. M. SMITH and B. A. D. STOCKER
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When one genetically marked sub-line of *Salmonella typhimurium* LT2 transmits colicine factor I to another sub-line at high frequency (10^{-1}) rare recombinants in respect of various chromosomal genes are produced. If colicine factor E1 is also being transmitted their frequency is increased fifty-fold, to c. 10^{-6}.

In such recombination pairs of genes which can be co-transduced by phage PLT22 usually remain coupled; if recombination results from transfer of part only of one chromosome into a " merozygote " (cf. *Hfr* mating in *Escherichia coli* K-12) the fragment transferred must be larger than that carried by a transducing phage particle.

When the parent strains differ in many characters recombinants selected for possession of one selective marker from each parent may be of many different types in respect of other markers. This suggests that at least some recombinants arise by recombination between two complete chromosomes. The relative frequency of various recombinant classes has been used to map four nutritional loci (*adC*, *proA*, *metA* and *tryB*), streptomycin-resistance, the H_1 and H_2 flagella antigen loci and two loci for production of flagella; the arrangement sought was that which accounted for the common recombinant classes by a minimum number of cross-overs. The map obtained is a single line or loop.

COLICINOGENY-CONFERRED FERTILITY IN *ESCHERICHIA COLI* K-12

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In *Escherichia coli* K-12, genetic transfer is mediated by the presence of a fertility factor, *F*, which determines the donor state. In *Salmonella*, Ozeki and Stocker have recently shown that a similar donor state is initiated by the *recent* transfer of a colicinogenic factor, I. In K-12, this same colicinogenic factor promotes the donor state in strains devoid of *F*. The colicinogeny fertility system in K-12 differs from that in *Salmonella* in two main respects. Firstly, there is no enhancement of fertility when transfer of colicinogeny I is made to potential donors, already colicinogenic for a second colicine, E1. Secondly, the donor state is readily stabilised and does not require recent transfer of colicinogeny I.

A comparison has been made of genetic transfer in K-12, mediated either by *F* (in the F^+ state), or by colicinogeny I. Preliminary results show that the main difference is one of frequency, the *F* mediated cross being one hundred times as fertile as the corresponding I cross. Among the recombinants, seven unselected markers are transferred at similar frequencies, suggesting that the size of the chromosomal fragments transferred may be similar in both types of crosses.

A GENE DETERMINING PRESENCE OR ABSENCE OF ε-N-METHYL-LYSINE IN *SALMONELLA* FLAGELLAR PROTEIN

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R. P. AMBLER
Department of Biochemistry, University of Cambridge

Only twenty amino-acids are common in proteins, possibly because there are only twenty different amino-acid-specifying base-groups in DNA.

Salmonella flagella, of many different antigenic types, consist of the protein flagellin. Some, but not all, flagellins contain the uncommon amino-acid ε-N-methyl-lysine (NML). The antigenically distinct phase 1 and 2 flagellins produced by a diphasic *Salmonella* both contain, or both lack, NML.

The H_1 and H_2 genes determining, respectively, the phase 1 and 2 flagellar antigens, were transduced between diphasic strains differing in NML character. The NML character of both the flagellins of a recombinant was that of the parent strain which provided its H_1 gene. This indicated that NML character was determined at H_1 or at a closely linked locus. By suitable crosses recombination between H_1 and NML was later obtained.

The H_1 and H_2 genes probably determine, respectively, the complete amino-acid sequences of phase 1 and 2 flagellins. The control of presence or absence of the uncommon amino-acid NML in a flagellar protein by a gene other than that which specifies the amino-acid sequence of the protein is compatible with the hypothesis that only twenty different amino-acid-specifying groups occur in DNA.

PAIRING INTERACTION AS A POSSIBLE BASIS FOR NEGATIVE INTERFERENCE

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The most plausible current theory of negative interference supposes that chromosome pairing is discontinuous and random, and that recombination occurs only within those small regions where effective pairing occurs. Thus recombination in two intervals would occur with greater than random frequency if these intervals

were so close as frequently to lie within one effectively paired region. The length of such regions has been estimated, in *Aspergillus* and phage, to be of the order 10^4 double nucleotides of DNA. The unique features of conjugation in *E. coli* K-12 enable distances between loci on the chromosome of the ♂ parent to be measured in absolute terms, independently of the recombination process. A study of the correlation between recombination in two intervals, in this system, has revealed a significant degree of negative interference which extends over a region of chromosome at least 10^6 double nucleotides long. Since there is evidence that effective pairing is unlikely to be continuous over such long regions, an interaction between different, small, regions of pairing is postulated to account for the effect. An explanation is offered of some anomalies between recombination data emerging from transductional and conjugal crosses involving the same strain and genetic loci.

MUTATIONS AFFECTING THE A MATING TYPE LOCUS IN *COPRINUS LAGOPUS*

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The *A* mating type locus of *Coprinus* controls the formation of clamp connections. Clamp bearing heterokaryons are only formed between strains with different *A* alleles. Mutations which enable a presumed haploid monokaryon to form clamp connections spontaneously were recovered from fruit bodies produced by common *A* heterokaryons. Similar mutants were also recovered among recombinants in crosses involving markers closely linked with the *A* locus.

Three out of four mutations tested are either very close to the two sub-units of the *A* locus or involve one of them. Stocks carrying them show no detectable *A* incompatibility reaction and will dikaryotize all *A* tester stocks. The fourth is a mutation at an unlinked locus. The mutant gene appears to be a recessive suppressor since stocks carrying it are unable to dikaryotize common *A* tester stocks.

THE INHERITANCE AND SURVIVAL OF THE XASTA (*Xa*) MUTANT OF *DROSOPHILA MELANOGASTER*

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School of Agriculture, University of Nottingham

The Xasta condition in *D. melanogaster* is determined by a semi-dominant wing mutation which is lethal when homozygous. The heterozygote has a wing with a deeply cut edge. Early cytological analysis showed that the condition was inseparable from a translocation between the right arms of chromosomes 2 and 3 and that two inversions were also present.

The present investigation has considered the results of single crosses involving Xasta and its survival in small population cages with or without the inversions. The results clearly demonstrate the existence of a state of balanced polymorphism in the population, probably controlled by the inversions.

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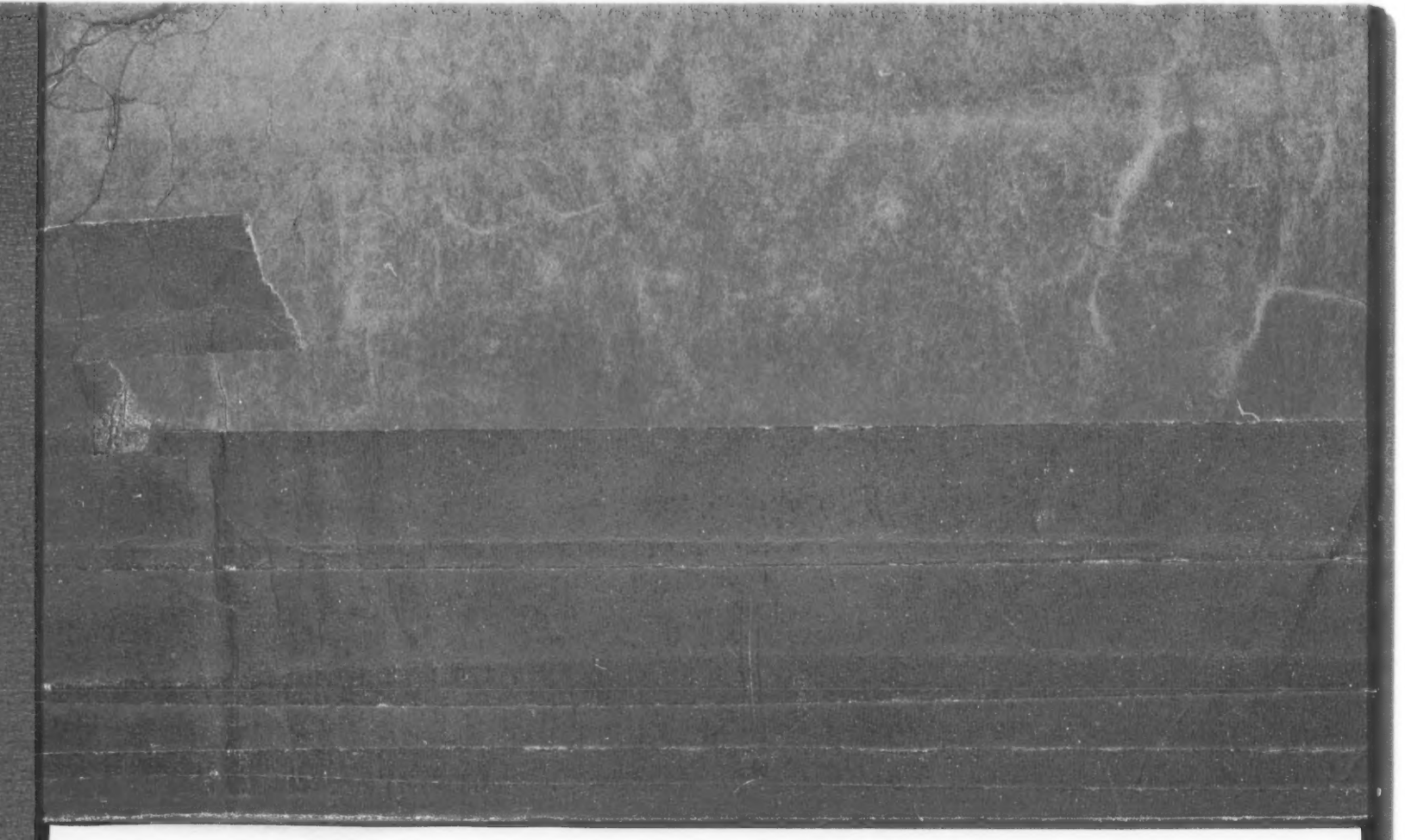
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